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<p>(21) International Application Number: PCT/GB96/02061</p> <p>(22) International Filing Date: 23 August 1996 (23.08.96)</p> <p>(30) Priority Data: 9517263.1 23 August 1995 (23.08.95) GB</p> <p>(71) Applicant (for all designated States except US): CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED [GB/GB]; Cambridge House, 6-10 Cambridge Terrace, Regent's Park, London NW1 4JL (GB).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): COLLINS, Mary, Katharine, Levinge [GB/GB]; Flat 4, Philips House, 52 Goodge Street, London W1P 1FP (GB). WEISS, Robin, Anthony [GB/GB]; 25 Cyprus Avenue, London N3 1SS (GB). TAKEUCHI, Yasuhiro [JP/GB]; 141 Elborough Street, London SW18 5DS (GB). COSSET, François-Loïs [FR/FR]; 32, rue L.-Thévenet, F-69004 Lyon (FR).</p> <p>(74) Agents: CALDERBANK, T., R. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
<p>(54) Title: EXPRESSION SYSTEMS</p> <p><i>pol gene... TCT AGA CTG ACA TGG CCG GTT CAA CGC TCT CAA AAC CCC TTA AAA ATA AGG TTA ACC CGC GAG GCC CCC TAA tcccttaatctctgalgttcagaggggcgtac tgcgtcgccgggtccatgtcgccggccacccggccacc ATG AAA ACA TTT AAC ATT TCT... bsr gene</i></p> <p>Schematic structure of CeB expression vector</p> <p>(57) Abstract</p> <p>The invention relates to new expression systems and in particular to an expression system in which a gene of interest is expressed at an optimal level. The invention provides a recombinant expression vector comprising a gene of interest and a selectable marker gene, wherein the selectable marker gene is arranged downstream of the gene of interest and a stop codon associated with the gene of interest is spaced from a start codon of said selectable marker gene at a distance which is sufficient to ensure that translation reinitiation is required before said selectable marker protein is expressed from the corresponding mRNA. Examples of such expression systems are vector viral packaging cell lines and a number of preferred cell lines have been identified.</p>			

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Expression systems

5 The present invention relates to new expressions systems, and in particular to expression systems in which a gene of interest is expressed at an optimal level. Particular examples of such expression systems are retroviral packaging cell lines and a number of preferred cell lines have been identified.

10 The ability of eukaryotic and prokaryotic ribosomes to reinitiate translation at an internal start codon within an mRNA sequence has previously been recognised. Studies have been reported in which the efficiency of the process, which 15 is generally regarded as being low, has been connected with the length of the intercistronic sequence (Kozak (1987) Mol. Cell Biol. 7, 3438-3445). Selection of this sequence or spacer as 70bp in length, and containing no other start codons, has been previously reported as being optimal for 20 reinitiation in a eukaryotic cell line (Cosset F-L., Virology (1991) 185, 862).

25 The applicants have found a way in which the inefficiency associated with the translation reinitiation process can be used to good effect.

According to the present invention there is provided a recombinant expression vector comprising a gene of interest and a selectable marker gene, wherein the selectable marker gene is arranged downstream of the gene of interest and a stop codon associated with the gene of interest is spaced 30 from a start codon of said selectable marker gene at a distance which is sufficient to ensure that translation reinitiation is required before said selectable marker protein 35 is expressed from the corresponding mRNA.

The invention further provides a process for producing cell lines in which a gene of interest is expressed, which process comprises transforming host cells with an expression vector comprising said gene of interest and a selectable marker gene, wherein the selectable marker gene is arranged downstream of the gene of interest and a stop codon associated with the gene of interest is spaced from a start codon of said selectable marker gene at a distance which is sufficient to ensure that translation re-initiation is required before said selectable marker protein is expressed from the corresponding mRNA, and selecting those cells where expression of the selectable marker gene may be detected.

Since re-initiation of translation is a relatively inefficient process, this means that the selectable marker protein will be expressed at lower levels than the product of the gene of interest. When the marker protein is expressed at detectable levels, the gene of interest will be expressed at higher levels. This will ensure that during the subsequent selection procedure, only those cell clones which express the gene of interest at higher or optimal levels will survive. Low expressing clones will be eliminated by the selection process.

Cells transformed with the above-described expression vectors form a further aspect of the invention.

The host cells are suitably eukaryotic or prokaryotic host cells, preferably eukaryotic host cells.

The number of nucleotides in the space between the stop codon of the gene of interest and the start codon of the selectable marker will suitably be in the range of from 20-200 nucleotides, preferably from 60-80 nucleotides, even more preferably 70-80 nucleotides.

The vectors used in the process of the invention may be any of the known types, for example expression plasmids or viral vectors.

5 Selected cells may be cultured and if required, the protein product of the gene of interest isolated from the culture using conventional techniques. Alternatively, expression of the gene of interest may result in other desired effects, for example, where the gene of interest is included as part
10 of a viral packaging construct.

Some experimental and clinical gene transfer protocols require the design of gene transfer vectors suitable for *in vivo* gene delivery (Miller, A.D. 1992. *Nature* 357:455-460). Retroviral vectors are attractive candidates for such applications, because they can provide stable gene transfer and expression (Samarut J. et al., *Meth. Enzymol.* in press) and because packaging cells have been designed which produce non-replication competent viruses (Miller A.D (1990) *Hum Gene Ther.* 1 5-14). However currently available recombinant retroviruses suffer from a number of drawbacks.

Packaging cell lines provide in trans the retroviral proteins encoded by the gag, pol, and env genes required to obtain infectious retroviral particles. The gag and pol products are respectively the structural components of the virion cores and the replication machinery (enzymes) of the retroviral particles whereas the env products are envelope proteins responsible for the host-range of the virions and for the initiation of infection and for sensitivity to humoral factors. An ideal packaging cell line should produce retroviruses that only contain the retroviral vector genome, and absolutely no replication-competent genomes or defective genomes encoding some of the viral structural genes.

A number of packaging cell lines designed for human gene transfer have been designed in the past by introducing plasmid DNAs which contain "helper genomes" encoding gag, pol and/or env genes into cells.

5

Retroviral packaging cell lines are cells that have been engineered to provide in trans all the functions required to express infectious retroviral vectors. A helper genome (or construct or unit), is herein also referred to as "retroviral packaging construct (or unit)" or "packaging-deficient construct (or genome unit)" or "gag-pol/env expression plasmids".

10 Much efforts has been made to design strategies to optimize the helper-genomes in order (i) to get the highest production of retroviral packaging functions (which correlates which infection titers of retroviral particles) and (ii) to minimise the chance that the helper genome can be transmitted via the viral particles (which may lead to 15 emergence of unwanted retroviral forms).

20 The first of these packaging cell lines used full length retroviral genomes as helper genomes that had been crippled for important cis-regulated replicative functions (reviewed 25 in Miller, Hum. Gene. Ther. 1:5-14 1990). In order to reduce the possibility of occurrence of replication-competent viruses and of transfer of virus structural genes, a second generation of safer packaging cell lines has been designed by using two separate and complementary helper genomes which 30 express either gag-pol or env and are packaging-deficient (Miller supra).

35 The cells into which these helper genomes were introduced were isolated by cotransfected them with plasmids encoding selectable markers. However, as no selection was applied on

the packaging-deficient retroviral genome itself, the helper functions can be lost during the passages of the cells in culture and the current packaging systems provide limited titers of infectious retroviral vectors, usually only of the 5 order of 10^5 - 10^6 infectious units i.u/ml. Indeed the cotransfection with a plasmid encoding a selectable marker does not directly select the best gag-pol-env-expressing cells.

10 The invention further provides a retroviral packaging cell line comprising a host cell transformed with (i) a packaging deficient construct which expresses a viral gag-pol gene and a first selectable marker gene, and/or (ii) a packaging-deficient construct which expresses a viral env gene and a 15 second selectable marker gene; wherein a start codon of the first and second selectable markers are spaced from the stop codons of the viral gag-pol gene and the viral env gene respectively by a distance which ensures that reinitiation of mRNA translation is required for expression of marker 20 protein product of said first and/or second selectable marker gene.

25 The retroviral packaging cell line may be obtained by the above described process which will involve selecting transfected cells which express said first and/or second marker genes.

30 By using helper constructs which are directly selectable and which provide for high expression of the viral gene, high titre retroviral vectors may be obtained.

Helper constructs for use in the process form a further aspect of the invention.

35 The retroviral vectors prepared from the conventional

packaging cell lines are usually not contaminated by replication-competent retroviruses (RCRs). However, recombinant amphotropic murine retroviruses have been shown to arise spontaneously from certain packaging cell lines.

5 The generation of such RCRs involves recombination at least between gag-pol/env packaging sequence and vector sequences (Cosset et al., *Virology*, (1993) 193:385-395).

10 Recombinant RCRs have been associated with the development of lymphomas in some severely immunosuppressed monkeys (Donahue et al., *J. Exp Med* (1992) 176: 1125-1135). In addition, retroviral vector preparations may also contain, at low frequencies, retroviruses coding for functional envelope glycoproteins (Kozak and Kabat, 1990, *J. Virol.* 64: 15 3500-3508) or for gag-pol proteins. Although the pathogenicity of these gag-pol or env recombinant retroviruses is probably low, more evolved recombinant retroviruses with higher pathogenic potential may occur when injected in vivo, by recombination and/or complementation of 20 the initial recombinant viruses with some endogenous retroviruses.

25 In a preferred embodiment of the retroviral packaging cell lines of the invention, the overlapping sequences between the genomes of the retroviral vector and the helper construct are reduced, for example as compared to constructs such as CRIPenv and CRIPAMgag (Danos et al., *Proc. Natl. Acad. Sci USA* 85: 6460-6464). In particular, the viral sequences in the helper construct are reduced, for example, 30 not only the packaging sequence but also the 3' Long Terminal Repeat (LTR), the 3' non-coding sequence and/or the 5'LTR may be eliminated.

35 The possibility of generation of such RCRs and recombinant retroviruses can be reduced by reducing the overlapping

sequences between the genomes of both the retroviral vector and the helper construct.

Conventional retroviral vectors are strongly inactivated by 5 human serum which makes them of limited or no use for in situ gene transfer in gene therapy applications. It has previously been shown that inactivation by complement in human serum is controlled by the cell line used to produce the virions and by viral envelope determinants (Takeuchi et 10 al., J. Virol (1994) 68:8001-8007). In particular, inactivation is caused by some properties of the cell lines that have been used to construct the packaging cells (NIH-3T3) and also by viral determinants located in the retroviral envelope as shown (Takeuchi et al., J. Virol 15 (1994) 68:8001-8007). In vivo gene delivery is an important goal for a number of human gene therapy strategies.

The applicants have found that certain cell lines form 20 preferred packaging cell lines.

Particularly preferred packaging cell lines are the HT1080 line, the TE671 line, the 3T3 line, the 293 line and the Mv-1-Lu line. One example of retroviral packaging cells that 25 will produce complement-resistant virus comprise human HT1080 cells and express RD114 envelope. Such cells form a preferred aspect of the invention.

Packaging cell lines according to the invention provide 30 50-100 fold increased titers of retroviral vectors as compared to conventional packaging cell lines. Retroviral vectors provided by these new cells are safe, in terms of generation of RCRs, and considerably more resistant to inactivation by human complement.

35 Packaging cell lines according to the invention may be able

to transduce helper-free, human complement-resistant retroviral vectors at titers consistently higher than 10^7 i.u./ml.

5 Suitable semi-packaging cell lines in accordance with the invention are those which express only the gag-pol genes. Such cell lines may suitably be derived from TE671, MINK Mv-1-Lu, HT1080, 293 or NIH-3T3 cells by introduction of plasmid CeB (the MoMLV gag-pol expression unit).

10 Particularly preferred expression vectors in accordance with the invention for use in retroviral packaging cell lines are those which include MLV gag and pol genes such as CeB. Other plasmids may include gag and pol genes from other 15 retroviruses or chimeric or mutated gag and pol genes.

20 Various viral and retroviral envelope genes may be included in the plasmids such as MLV-A envelope, GALV envelope, VSV-G protein, BaEV envelope, RD114 envelope and chimeric or mutated envelopes. Plasmids which include the RD114 env gene such as FBdelPRDSAF as illustrated hereinafter, provide 25 one example of suitable constructs.

25 The novel retroviral packaging cells described hereinafter, have been designated FLY cells, and may be designed for in vivo gene delivery.

30 Considerable variations were found between the various cell lines screened for their ability to release type C mammalian retroviruses. In addition, few cell lines were able to produce retroviruses completely resistant to human complement. Based on these two criteria, human fibrosarcoma HT1080 and rhabdomyosarcoma TE671 cells were selected for optimum construction of packaging cells.

Other studies have shown the importance of endogenous retrovirus expression in the generation of recombinant retroviruses from retroviral packaging lines (Ronfort et al., *Virology*, (1995), 207, 271-275, Vanin, E.F. et al., *J Virol* (1994) 68:4241-4250.). The co-packaging of an endogenous genome and a vector can lead to emergence of recombinant retroviruses (Vanin et al., *supra*). Recombination involves template switching during reverse transcription of such hybrid retroviruses (Hu et al., *Science*, (1990) 250:1227) and homologies between the two genomes considerably enhance the frequency of reverse transcriptase jumps (Zhang et al., *J. Virol.* (1994) 68: 2409-2414). Therefore an ideal packaging cell line should not express endogenous MLV-like (or type C retrovirus-like) retroviral genomes which can be packaged by type C gag proteins (Scadden et al., *J. Virol.* (1990) 64: 424-427, Torrent et al., *J. Mol. Biol.* (1994) 240 434-444).

Packaging of human endogenous retroviral RNA was not detected in TELCeB and FLY packaging cells when virion associated RNA was analysed by RT-PCR using generic primers. HT1080- and TE671 derived packaging cell lines may be safer in this respect than those generated from NIH3T3 cells, such as GP+EAM12 cells, which are known to express and package sequences related to type C retroviruses (Scadden et al. *supra*).

To generate the FLY packaging cell lines, HT1080 cells were transfected with gag-pol and env expression plasmids designed to optimise viral protein expression. Direct selection for viral gene expression was achieved in accordance with the invention by expression of a selectable marker gene by re-initiation of translation of the mRNA expressing the viral proteins. This strategy resulted in packaging cell lines capable of producing extremely high

5 titer viruses. Furthermore, long-term expression of packaging functions can be maintained in these cells. Many unnecessary viral sequences were eliminated from the packaging constructs to reduce the risk of helper virus generation; indeed the final packaging cells did not produce helper virus, in that no replication competent virus (RCR) could be detected per 10^7 vector particles.

10 The FLY packaging cells described herein are safer than, for example, psiCRIP cells, at least for generation of env recombinant retroviruses as is illustrated in Table 4 hereinafter, probably because less retroviral sequences overlapping with the vector were present in the present env-expression plasmid. Few reports have addressed the question 15 of the characterization of recombinant retroviruses (RVs) (Cosset, F.L., et al., Virology (1993) 193:385-395). It is possible that such RVs could not be detected in previous packaging cell lines due to lower overall titers. RVs are defective in normal cell culture conditions but are likely 20 to evolve to replication competent viruses if they are allowed to replicate in cells complementing their expression like co-cultivated packaging cells (Bestwick et al., Proc. Natl Acad Sci USA, (1988) 85: 5404-5408, Cosset et al., (1993) *supra*).

25

In preferred retroviral packaging systems according to the invention, RVs are eradicated for example by removal of viral LTRs from the packaging construct.

30

Consistent with our previous studies (Takeuchi, Y., et al., J Virol (1994) 68:8001-8007), LacZ(RD114) and lacZ(MLV-A) pseudotypes produced from HT1080 and TE671 cells were more resistant to human complement than LacZ(RD114) or LacZ(MLV-A) pseudotypes produced by 3T3 of dog cells. It was 35 therefore decided to use RD114 and MLV-A env genes to

generate recombinant virions with MoMLV cores.

The sequence of RD114 env gene was determined and is shown in Figure 4. It was found to be very close to BaEV (baboon 5 endogenous virus) a type C retrovirus (Benveniste, R.E. et al., Proc. Natl. Acad. Sci. USA (1973) 70:3316-3320; Kato, S. et al., Japan. J. Genet. (1987) 62:127-137) with an envelope gene displaying similarities to the external part of type D simian retroviruses (SRVs). RD114 uses the SRV 10 receptor on human cells (Sommerfelt & Weiss, Virology (1990) 176:58-69; Sommerfelt, M.A. et al., J Virol (1990) 64:6214-6220) making the FLY packaging cells with RD114 envelope capable of generating virions with different tropism. Retroviral vectors prepared so far for human gene 15 therapy have used either MLV-A or GALV (gibbon ape leukemia virus) envelopes which display some similarities (Battini, J.L., et al., J Virol. (1992) 66:1468-1475) and which use two related cell surface receptors for infection (Miller, D.G. et al., J Virol (1994) 68:8270-8276). Differences in tissue- 20 specific expression of MLV-A or GALV receptors have been reported (Kavanaugh et al., Proc Natl Acad Sci USA (1994) 91:7071-7075).

The invention will now be particularly described by way of 25 example with reference to the accompanying drawings in which:

Figure 1. illustrates the structure and expression of CeB. The env gene (XbaI-Clal) of plasmid pCRIP was removed and 30 was replaced by coinsertion of the two fragments XbaI-Sfil (restriction sites underlined) from pOXEnv and a Sfil-Clal PCR product containing the bsr selectable marker. This results in positioning the bsr start codon (shadowed) 74 bp downstream to the pol stop codon (bold).
35

5 Open triangle are start codons (gag and bsr), black triangles are stop codons (pol and bsr). The shadowed triangle is the start codon of env, in the same reading frame with that of bsr. SD and SA are the splice donor and splice acceptor sites.

Figure 2 illustrates the structure and expression of FbdelPASAF.

10 Immediately after the stop codon of env (bold) was inserted a non retroviral Kasl-Ncol (restriction sites underlined) linker which positions the phleo start codon (shadowed) 76 bp downstream.

15 Open triangle are start codons (env and phleo), black triangles are stop codons (env and phleo). SD and SA are the splice donor and splice acceptor sites.

Figure 3 illustrates plasmids for expression of Ampho, Eco, RD114, Xeno, 10A1, GALV, VSV-G and FeLVb envelopes.

20 All genes are expressed in the same backbone as detailed in fig. 2. The BglII sites for ecotropic (MoMLV strain), 10A1, xenotropic (NZB.1.V6 strain) and amphotropic (4070A strain), the Ndel site of RD114 (SC3C strain, the BamH1 site for both FeLVb and GALV were used as 5' ends, and linked to Mscl site immediately after the splice donor site in the 25 leader of FB29 LTR.

Figure 4 shows the sequence of the RD114 env gene (SEQ ID No 1).

30 Figure 5 shows the genetic structure of gag-pol constructs. Initiation (\blacktriangleright) and termination (\blacktriangledown) codons are shown. The thick dotted line below each construct shows MLV-derived sequences. Nucleotide positions of MLV-derived sequences are shown according to: Shinnick et al. (1981) (from nt 1 to nt 35 6000 with deletion of the packaging signal (DY) from BalI

(nt 215) to PstI (nt 568), and with some further MoMLV sequences in both CeB and CeB DS- from nt 7676 to nt 7938. gag-pol and bsr genes were expressed from the same transcription unit using the either a retroviral promoter (Mo LTR) or a non retroviral promoter (hCMV) and non retroviral polyadenylation sequence (polyA). Splice donor (SD) and acceptor (SA) sites are indicated. The thin line denotes retroviral non coding sequences. The thick line shows the rabbit beta-1 globin intron B. The position of some restriction sites is indicated.

The nucleic acid sequences of portions of constructs (as shown in Figure 5 (boxed areas)) are displayed for CeB (SEQ ID No 2, Figure 6), hCMV+intron (SEQ ID No 3, Figure 7) and hCMV+intronkaSD (SEQ ID No 4, Figure 8).

The nucleic acid sequences of portions of constructs (as shown in Figure 3 (boxed areas)) are displayed for FbdelPASAF (SEQ ID No 5, Figure 9), FbdelPMOSAF (SEQ ID No 6, Figure 10), FbdelPGASAF (SEQ ID No 7, Figure 11), FbdelPRDSAF (SEQ ID No 8, Figure 12) and CMV10A1 (SEQ ID No 9, Figure 13) are shown.

The components of the viral particles are produced by two independent expression plasmids (gag-pol or env) which also contain selectable markers (bsr or phleo) expressed from the same transcriptional units as gag-pol or env (figs. 1& 2). The selectable markers are located downstream to gag-pol or env genes and there is an optimal distance between the stop codon of the upstream reading frames and the start codon of the selectable genes that should allow re-initiation of translation (Kozak, Mol Cell Biol. (1987) 7:3438-3445). Because there is no "Kozak" sequence (Kozak, Cell, (1986) 44: 283-292) required for a normal initiation of translation for

the marker gene, they can only be expressed by re-initiation of translation after the upstream viral gene has been successfully expressed. Consequently and also because re-initiation of translation is a poorly efficient process, after transfection of these plasmids, cells resistant to the drugs corresponding to those selectable genes express high levels of the viral proteins.

To avoid viral transmission of these "helper" genomes the constructs used suitably have the classical deletions of both the packaging sequence located in the leader region and of the 3'LTR, the latter being replaced by SV40 polyadenylation sequences (Figs 1 & 2).

Plasmid CeB is the MoMLV gag-pol-expression unit. It derives from pCRIP, a plasmid used to generate the constructs introduced in the CRIP and CRE packaging cell lines (Danos and Mulligan, 1988). As shown in fig. 1 for generation of plasmid CeB the env gene of pCRIP has been deleted mostly and the bsr selectable marker, -encoding a protein conferring resistance to blasticidin (Izumi et al., Experimental Cell Research (1991) 197, 229-233)- has been inserted downstream to pol gene. There are exactly 74 bp with no ATG triplets between the stop codon of pol and the start codon of bsr, this allows its expression by re-initiation of translation on the gag-pol mRNA, after translation of the gag-pol reading frame.

Fbde1PASAF is a plasmid expressing the amphotropic env gene and the phleo selectable marker conferring resistance to phleomycin (Gatignol et al., FEBS Letters (1988) 230:171-175). By using a PCR-mediated mutagenesis strategy which modifies the end of env gene (see fig. 2), a 76 bp linker was inserted between the stop codon of env and the start codon of phleo. This allows expression of phleo from the

env mRNA by re-initiation of translation. In addition compared to known env-expressing constructs, this strategy of construction has reduced the length of sequences overlapping with the ends of conventional retroviral vectors. The env genes of Mo-MLV, FeLVb, NZB.1V6, 10A1, GALV and RD114 are expressed by plasmids FBdelPMoSAF, FBdelPBSAF, FBdelXSAF, FBdelpGSAF, FBdelp10A1SALF and FBdelPRDSA, respectively, by using the same backbone as FBdelPASAF (fig. 3). Retroviral vectors produced with the RD114 envelope will be useful for in vivo gene delivery as comparatively to MLV ecotropic or amphotropic envelopes, virions pseudotyped with RD114 envelopes are not inactivated by human complement when they are produced by Mink Mv-1-Lu cells or by some human cells (Table 1).

15

The HT1080 cell line, isolated from a human fibrosarcoma (ATCC CCL121). The TE671 cell line isolated from a human rhabdomyosarcoma (ATCC CRL 8805) (purchased from ATCC, and tested for absence of usual cell culture contaminants by ECACC), has been used for the definitive construction of packaging cell lines. HT1080 line was chosen among a panel of primate and human lines because MLV-A and RD114 efficiently rescued retroviral vectors from these cells and also because RD114 pseudotypes produced by this cell line were stable when incubated in human serum. In a standard assay (Takeuchi et al., J Virol (1994), 68, 8001-8007), these latter viruses were found more than 500 fold more stable than similar pseudotypes produced in 3T3 cells.

30

Another advantage for the use of non murine cells to derive packaging lines is the absence of MLV-related endogenous retroviral-like sequences (like VL30 in 3T3 cells) that can cross-package with MLV-derived retroviral vectors (Torrent et al., 1994) and generate potentially harmful recombinant retroviruses.

35

The helper constructs were introduced into other cell lines (HT1080 (table 2) Mink Mv-1-Lu (table 2), 3T3 (not shown), TE671 (table 2)) for the purpose of comparisons of the efficiency of the constructs.

5

As illustrated hereinafter (Table 2), the reverse transcriptase (RT) activity (provided by expression of the pol gene) in cells transfected with CeB is significantly higher than that of the same cells transfected by the 10 parental plasmid pCRIP or that of cells chronically infected by MLV. This enhancement of viral gene expression is correlated with the titers of lacZ retroviral vectors when an envelope is provided in CeB-lacZ cells after comparison 15 with titers of lacZ pseudotypes of either replication-competent viruses or other helper-free packaging systems.

For the generation of final packaging cell lines, the best 20 clonal env transfecants have been selected. Packaging systems obtained in this way will be able to produce helper-free retroviral vectors at titers greater than 10^8 infectious particles per ml, which would be 10-100 fold higher to helper-free preparations of others.

Because of the way the selectable markers are expressed (see 25 above), growing the packaging cells in phleomycin and blasticidin selective pressure increase and stabilize the expression of the retroviral components and particularly the envelopes, as it is possible that env glycoproteins have toxic effects for the producer cells in the long term which 30 may lead to a decrease of expression.

Such an enhancement of viral production observed with the 35 packaging systems described herein might increase the emergence of unwanted retroviruses having recombined between the genomes of both the retroviral vector and either of the

two packaging-deficient constructs. However, the constructs have been designed in such a way that it reduces the probability of emergence of recombinant viruses compared to the parental constructs. To check their safety, attempts 5 have been made to detect the presence of replication-competent retroviruses by a mobilisation assay of a lacZ provirus. No RC viruses have been found in all retroviral vector preparations tested so far.

10 The following Examples illustrate the invention.

Example 1

Preparation of Cell lines and viruses.

15 The following cell lines were used:

A204 (ATCC HTB 82), HeLa (ATCC CCL2), HT1080 (ATCC CCL121), MRC5 (ATCC CCL171), T24 (ATCC HTB 4), VERO (ATCC CCL81) and D17 (ATCC CCL183) were purchased from ATCC.

20 HOS, TE671 and Mv-1-Lu cells and their clones harboring MFGnlslacZ retroviral vector as described by Takeuchi et al., J Virol (1994), 68, 8001-8007.

25 The above cell lines were grown in DMEM (Gibco-BRL, U.K.) supplemented with 10% fetal calf serum.

EB8 (Battini et al., J. Virol (1992) 66: 1468-1475);
psiCRE, psiCRELLZ and psiCRIP (Danos et al., Proc. Natl. Acad. Sci USA (1988) 85: 6460-6464);
30 Cells GP+EAM12 (Markowitz et al., Virology (1988), 167, 400-406); and
NIH-3T3 murine fibroblasts.

These cell lines were grown in DMEM (GIBCO-BRL, U.K.)
35 supplemented with 10% new-born calf serum.

Mv-1-Lu, TE671 and HT1080 cells were transfected using calcium-phosphate precipitation method (Sambrook et al., "Molecular Cloning" 1989, Cold Spring Harbour Laboratory Press: New York) as described elsewhere (Battini et al., supra). CeB-transfected Mv-1-Lu, TE671 and HT1080 cells were selected with 3, 6-8 and 4 µg/ml of blasticidin S (ICN, UK), respectively, and blasticidin-resistant colonies were isolated 2-3 weeks later. Cells transfected with the various env-expression plasmids were selected with phleomycin (CAYLA, France): 50 µg/ml (for FBASALF-transfected cells) or 10 µg/ml (for FBASAF-, FbdelPASAF-, FbdelPMOSAF, FBdelPIOAISAF or FBdelPRDSAF-transfected cells). Phleomycin-resistant colonies were isolated 2-3 weeks later.

15 Production of lacZ pseudotypes using replication competent viruses, amphotropic murine leukemia virus (MLV-A) 1504 strain and cat endogenous virus RD114, was carried out as described previously (Takeuchi et al., J Virol (1994), 68, 8001-8007).

20

Example 2**Preparation of Plasmids.**

25 The env gene of pCRIP (Danos et al., supra) was excised by HpaI/ClaI digestion. A 500 bp PCR-generated DNA fragment was obtained using pSV2-bsr (Izumi et al., Experimental Cell Research (1991), 197, 299-233) as template and a pair of oligonucleotides:

30 (5' >CGGAATTCGGATCCGAGCTCGGCCAGCCGCCACCATGAAACATTTAACATTTC
TC) (SEQ ID NO 2) at 5' end and
(5' >GATCCATCGATAAGCTTGGTGGTAAAACTTT) (SEQ ID No 3) at 3' end, with SfiI and ClaI sites, respectively. This fragment was inserted in HpaI/ClaI sites of pCRIP by co-ligation with a 85 bp HpaI/SfiI DNA fragment isolated from pOXEnv (Russell et al., Nucleic Acids Research (1993), 21, 1081-1085) which

provides the end of the Moloney murine leukemia virus (MoMLV) pol gene. The resulting plasmid named CeB (Fig. 1) could express the MoMLV gag-pol gene as well as the bsr selectable marker conferring resistance to blasticidin S, 5 both driven by the MoMLV 5'LTR promoter.

A series of env-expression plasmids was generated using the 4070A MLV (amphotropic) env gene (Ott et al., J Virol (1990), 64, 757-766) and the FB29 Friend MLV promoter 10 (Perryman et al., Nucleic Acid Res (1991), 19, 6950). In FBASALF (Fig. 1) a BglII/ClaI fragment containing the env gene was cloned in BamHI/ClaI sites of plasmid FB3LPh which also contained the C57 Friend MLV LTR driving the expression 15 of the phleo selection marker. A 136 bp env fragment was generated by PCR using plasmid FB3 (Heard et al., J Virol (1991), 65, 4026-4032) as template and a pair of oligonucleotides: (5'>GCTCTTCGGACCCTGCATTC) (SEQ ID NO 4) at 5' end (before ClaI site) and (5'>TAGCATGGCGCCCTATGGCTCGTACTCTATAGGC) (SEQ ID NO 5) at 3' 20 end, providing a KasI restriction site immediately after the env stop codon. This PCR fragment was digested using ClaI and KasI. A DNA fragment containing the FB29 LTR and the MLV-A env gene was obtained by NdeI/ClaI digestion of FBASALF. The fragments were co-ligated in NdeI/KasI digested 25 pUT626 (kindly provided by Daniel Drocourt, CAYLA labs, France). In the resulting plasmid, named FBASAF (Fig. 1), the phleo selectable marker was expressed from the same mRNA as the env gene. A BglII restriction site was created after the MscI site at position 214 in the FB29 leader by using a 30 commercial linker (Biolabs, France). A NdeI/BglII fragment containing the FB29 LTR was co-inserted with the BglII/ClaI env fragment in NdeI/ClaI-digested FBASAF plasmid DNA, resulting in plasmid FBdelPASAF (Fig. 1). Compared to FBASAF, FBdelPASAF has a 100bp larger deletion in the leader 35 region.

Example 3

Cloning and Sequencing of the RD114 env gene
The RD114 env gene was first sub-cloned in plasmid
5 Bluescript KS+ (Stratagene) as a 3 Kb HindIII insert
isolated from SC3C, an RD114 infectious DNA clone (Reeves et
al., J. Virol (1984), 52, 164-171). A 2.7 kb Scal-Hind III
fragment of this subclone containing the RD114 env gene was
sequenced (Figure 4 (SEQ ID NO 1)- EMBL accession number;
10 X87829). The 5' non-coding sequence upstream of an NdeI site
was deleted by an EcoRI/NdeI digestion followed by filling-
in with Klenow enzyme and self-ligation. From this plasmid,
two DNA fragments were obtained: a BamHI/NcoI 2.5 Kb
fragment and a 63 bp PCR-generated DNA fragment using
15 (5'>CGCCTCATGGCCTTCATTAA) (SEQ ID NO 6) at 5' end (before
NotI site) and (5'>TAGCATGGCGCCTCAATCCTGAGCTTCTTCC) (SEQ ID
NO 7) at 3' end, providing a Kasi restriction site just
after RD114 env gene stop codon. The PCR fragment was
digested with NcoI and Kasi. Both fragments were co-
20 inserted between BglII and Kasi sites of FBdelPASAF and the
resulting plasmid was named FBdelPRDSAF (Fig. 1).

Plasmid pCRIPAMgag- (Danos, O. et al., Proc Natl Acad
Sci USA (1988) 85:6460-6464) was used for transfection.

25 Example 4

Infection assays.

Target cells were seeded in 24-multiwell plates (4×10^4 cells
per well) and were incubated overnight. Infections were then
30 carried out at 37°C by plating 1 ml dilutions of viral
supernatants in the presence of 4 μ g/ml polybrene (Sigma) on
target cells. 3h later virus-containing medium was replaced
by fresh medium and infected cells were incubated for two
days before X-gal staining, performed as previously
35 described (Tailor et al., J Virol (1993), 67, 6737-6741,

Takeuchi et al., J Virol (1994), 68, 8001-8007). Viral titers were determined by counting lacZ-positive colonies as previously described (Cosset et al., J. Virol. (1990) 64: 1070-1078). Stability of lacZ pseudotypes in fresh human 5 serum was examined by titrating surviving virus after incubation in 1:1 mixture of virus harvest in serum-free medium and fresh human serum for 1 h at 37°C as described before (Takeuchi et al. supra).

10 Example 5

Reverse transcriptase (RT) assay.

RT assays were performed either as described previously (Takeuchi et al. supra) or using an RT assay kit (Boehringer 15 Mannheim, U.K.) following the manufacturer's instruction but using MnCl₂ (2 mM) instead of MgCl₂.

Example 6

20 Screening producer cell lines.

Viral particles generated with RD114 envelopes have been found to be more stable in human serum than virions with MLV-A envelopes and that the producer cell line also controls sensitivity (Takeuchi et al. supra). A panel of 25 cell lines was screened for their ability to produce high titer viruses and for the sensitivity of these virions to human serum. To do this, cells were infected at high multiplicity with lacZ pseudotypes of either MLV-A or RD114 and cells producing helper-positive lacZ pseudotypes were 30 established. Human HT1080 and TE671 and mink Mv-1-Lu cells were found to release high titer lacZ(RD114) and lacZ(MLV-A) viruses. LacZ(MLV-A) pseudotypes produced by HT1080 cells were more resistant to human serum than those produced by other cells. The titer of these viruses was only four-fold 35 less following a 1 hr incubation with human serum than a

control incubation (Table 1). LacZ(RD114) pseudotypes produced by human cells or mink Mv-1-Lu cells were in general stable in human serum (Table 1). These results suggested that HT1080, TE671 and Mv-1-Lu cells provided the best combination of high lacZ titers and resistance to human serum and they were therefore used for the generation of retroviral packaging cells.

Table 1. Titer and stability of lacZ pseudotypes.

Producer cell	LacZ (MLV-A)		LacZ (RD114)	
	Titer ^a	Stability ^b	Titer ^a	Stability ^b
A204	650	<3	1,200	105
HeLa	9	nd	2,000	115
HOS	4,500	6	23,000	86
HT1080	2,000,000	26	400,000	129
MRC-5	450	10	1,000	nd
T24	350	nd	1,200	nd
TE671	15,000	2	90,000	38
VERO	260	nd	90	nd
D17	900	<1	200,000	1
Mv-1-Lu	80,000	1	200,000	120

a: titration on TE671 cells as lacZ i.u./ml

b: % of infectivity of human serum-treated viruses compared to fetal calf serum-treated viruses

Example 7

Construction of an improved gag-pol expression vector.

A MoMLV gag-pol expression plasmid, CeB (Fig. 1), was

derived from pCRIP (Danos et al., Proc. Natl. Acad. Sci. USA (1988) 85: 6460-6464). Approximately 2 Kb of env sequence were removed from pCRIP and the bsr selectable marker, conferring resistance to blasticidin S (Izumi et al., 5 Experimental Cell Research (1991) 197:229-233), was inserted 74 nts downstream of the gag-pol gene. This 74 nts interval had no ATG triplets and was thought to provide an optimal distance between the stop codon of the pol reading frame and the start codon of the bsr gene to allow re-initiation of 10 translation (Kozak Mol Cell Biol., 1987, 7: 3438-3445). There was no "Kozak" consensus sequence (Kozak Cell, (1986) 44: 283-292) at the 5' end of the marker gene. Therefore, bsr could only be expressed by re-initiation of translation after the upstream gag-pol gene had been expressed. 15 Consequently, after transfection of CeB in Mv-1-Lu/MFGnlsLacZ (ML), TE671/MFGnlsLacZ (TEL) or HT1080 cells, blasticidin S-resistant bulk populations and most cell clones expressed high levels of gag-pol proteins assessed by the reverse-transcriptase (RT) activity found in cell 20 supernatants (Table 2). Considerably higher RT activities were found in bulk populations of CeB-transfected ML cells compared to bulk population of ML cells stably transfected with the parental pCRIP construct. Similarly the RT activities of two packaging cell lines generated using 25 pCRIPenv- construct, psiCRE cells (Danos et al., *supra*) and EB8 cells (Battini *supra*) were less than that of CeB transfected clones (Table 2). Finally, RT activity in CeB transfected cell supernatants was higher than that of cells chronically infected by replication-competent MLV-A (Table 30 2).

Table 2. Secreted reverse transcriptase expression

Cell ^a	RT activity ^b	LacZ Titer ^c

ML/MLV-A	1	8x10 ⁴
MLSVB	0.1	<1
MLCRIP (bulk)	0.15	nd
MLCeB (bulk)	1.7	nd
5 MLCeB1	4.2	1x10 ⁶
MLCeB4	1.6	1x10 ⁶
TEL/MLV-A	3.6	2x10 ⁶
TELCeB6	5.2	4x10 ⁷
HT1080/MLV-A	1.1	1x10 ⁶
10 HTCeB6	1.9	1x10 ⁶
HTCeB18	2.7	2x10 ⁶
HTCeB22 (FLY)	6.9	5x10 ⁶
HTCeB48	5.5	3x10 ⁶
EB8	0.22	1x10 ⁴
15 psiCRE-LLZ	1.2	1x10 ^{5a}

a: ML, Mv-1-Lu cells harboring a MFGnlslacZ provirus; TEL, TE671 cells harboring a MFGnlslacZ provirus; /MLV-A, cells chronically infected with MLV-A 1504 strain; MLSvB, ML cells transfected with a plasmid pSV2bsr alone; MLCRIP, ML cells co-transfected with pCRIP and pSV2bsr.

20 b: Average of arbitrary units relative to ML/MLV-A RT activity of at least two independent experiments was shown. The standard errors did not exceed 20 % of the values.

25 c: titration on TE671 cells as lacZ i.u./ml. After polyclonal transfection of a plasmid which expresses MLV-A env in MLCeB clones, TELCeB clones, HTCeB clones and EB8 cells; nd, not done.

d: titration on NIH3T3 cells

30 To rescue infectious lacZ viruses, MLCeB and TELCeB clones were transfected with FBASALF DNA, a plasmid designed to express the MLV-A env gene (Fig. 1). Bulk populations of stable FBASALF transfectants were isolated and supernatants were titrated using TE671 cells as targets. Titers of lacZ viruses were higher than either MLV-A infected ML or TEL cells, or FBASALF-transfected EB8 cells (Table 2). These 35 data suggested that CeB was an extremely efficient MLV gag-pol expression vector in mink Mv-1-Lu and TE671 cells. CeB

was therefore used to derive packaging cells by transfection of HT1080 cells. 41/49 blasticidin S-resistant colonies had detectable levels of RT; 9 had RT activity higher than that of control MLV-A-infected HT1080 cells (data not shown).

5 Expression of gag precursor was confirmed in cell lysates and supernatants of these 9 HTCeB clones by immunoblotting using antibodies against p30-CA (data not shown). The 4 clones with the highest expression of gag proteins (clones 6,18,22 and 48) were infected at high-multiplicity with
10 helper free, lacZ pseudotypes bearing MLV-A envelopes (MFGn1slacZ(A)) produced by TELCeB6/FBASALF (Table 3) and then transfected with FBASALF. Supernatants of bulk, phleomycin-resistant transfectants were assessed for RT activity and lacZ titer (Table 2). Clone HTCeB22, named FLY,
15 was found to be the best gag-pol producer clone and was used to introduce env expression vectors for the generation of packaging cell lines.

Table 3. Titer following env construct transfection

	Producer cell	Env source	Titer ^a
5	psiCRIP lacZ 5	pCRIPAMgag-	6x10 ^{4b}
	GP+EAM12 lacZ 25	envAM	3x10 ^{5b}
10	TELCeB6	FBASALF ^c	5x10 ⁷
		FBASAF ^c	2x10 ⁷
		FbdelPASAF ^c	2x10 ⁷
15	TELCeB6	FbdelPASAF 1	3x10 ⁷
		FbdelPASAF 4	2x10 ⁷
		FbdelPASAF 6	1x10 ⁷
		FbdelPASAF 7	5x10 ⁷
		FbdelPASAF 8	1x10 ⁷
20		FbdelPRDSAF 2	1x10 ⁶
		FbdelPRDSAF 4	3x10 ⁵
		FbdelPRDSAF 7	1x10 ⁷
		FbdelPRDSAF 8	2x10 ⁶
25	FLY ^d	FbdelPASAF 1	1x10 ¹
		FbdelPASAF 4	1.5x10 ⁶
		FbdelPASAF 5	1x10 ⁶
		FbdelPASAF 7	1x10 ⁶
		FbdelPASAF 13	7x10 ⁶
		FbdelPASAF 14	4x10 ⁶
30		FbdelPASAF 15	1x10 ⁶
		FbdelPASAF 16	5x10 ⁶
		FbdelPASAF 17	6x10 ⁶
35	FLYA4 lacZ 3	FbdelPASAF 4	2x10 ^{7b}
40	FLY ^d	FbdelPRDSAF 1	2.5x10 ⁶
		FbdelPRDSAF 2	1x10 ⁷
		FbdelPRDSAF 6	5x10 ⁶
		FbdelPRDSAF 10	2x10 ⁶
		FbdelPRDSAF 11	3x10 ⁶
		FbdelPRDSAF 13	1x10 ⁶
		FbdelPRDSAF 17	5x10 ⁶
		FbdelPRDSAF 18	3x10 ⁷
45		FbdelPRDSAF 19	6x10 ⁶

Average titers of at least three independent experiments were shown. The standard errors did not exceed 30 % of the titer values.

50 a: titrated on TE671 cells as lacZ i.u./ml

b: results of best MFGnlslacZ producer clones.

c: bulk populations of env-transfectants in TELCeB6 cells.

d: titration after bulk infection with helper-free MFGnlslacZ.

5 Example 8

Construction of env expression vectors.

A series of MLV-A env expression plasmids were then generated (Fig. 1). In FBASALF, the env gene was inserted between two Friend-MLV LTRs, its expression driven by the FB29 MLV LTR (Perryman et al., *supra*). Most of the packaging signal located in the leader region was deleted. This plasmid also expressed the phleo selectable marker (Gatignol et al., *supra*) driven by the 3' LTR. FBASAF and FBdelPASAF were then designed following the same strategy used for CeB. These two vectors differed only by the extent of deletion of the packaging signal, FBdelPASAF having virtually no leader sequence. Compared to pCRIPAMgag- and pCRIPgag-2 env plasmids expressed in psiCRIP or psiCRE packaging cells (Danos et al., *supra*) about 5 Kb of gag-pol sequences was removed. In addition the 258 bp retroviral sequence containing the end of env gene and the begining of U3 found in pCRIPAMgag- and pCRIPgag-2 was also removed. For both FBASAF and FBdelPASAF plasmids, the phleo selectable marker was inserted downstream of the env gene by positioning a 76 nts linker with no ATG codons between the two open-reading frames. Phleo could therefore only be expressed by re-initiation of translation by the same ribosomal unit that had expressed the upstream env open reading frame. FBdelPASAF was also used to generate FBdelPRDSAF, an RD114 envelope expression plasmid (Fig. 1).

After transfection of the env plasmids into TELCeB6 cells (Table 2), bulk populations of phleomycin-resistant colonies were isolated and their production of lacZ virus measured

(Table 3). FBASALF gave a titer of 5×10^7 lacZ-i.u./ml, whilst titers with either FBASAF or FBdelPASAF were 2×10^7 lacZ-i.u./ml (Table 3). Titers of 5×10^7 or 10^7 lacZ-i.u./ml could be obtained with some FBdelPASAF cell clones or FBdelPRDSAF clones, respectively.

As FBdelPASAF has minimal virus-derived sequences and was shown to be the safest construct (see below and Table 4), it and FBdelPRDSAF were used to generate packaging lines from FLY cells (clone HTCeB22, Table 2). Envelope expression of these clones was assayed by interference to challenge with MFGnlslacZ(A) or MFGnlslacZ(RD) pseudotypes produced by TELCeB6/FBdelPASAF-7 or TELCeB6/FBdelPRDSAF-7, respectively (Table 3). The cell lines showing most interference were cross-infected at high multiplicity with these pseudotypes to provide MFGnlslacZ proviruses, and supernatants were then titrated on TE671 cells (Table 3). FLY-FBdelPASAF-13 (FLYA13 packaging line) and FLY-FBdelPRDSAF-18 (FLYRD18 packaging line) gave the highest productions of lacZ viruses, around 10^7 lacZ-i.u./ml. The best MFGnlslacZ producer clones derived from either psiCRIP cells (Danos et al., *supra*) or GP+EAM12 cells (Markowitz et al., *supra*) gave approximately 50 fold lower titers (Table 3). The lacZ titers of the FLY-derived lines shown in Table 3 are lower than the best TELCeB6-derived lines after transfection of either FBdelPASAF or FBdelPRDSAF (Table 3). However it should be noted that the lacZ provirus expressed in TELCeB6 cells was obtained after clonal selection but was introduced polyclonally in FLY-derived env-transfected cell clones. When FLY-FBdelPASAF-4 cells (FLYA4 packaging line), infected with helper-free MFGnlslacZ(RD), were cloned by limiting dilution the best clones (eg. FLYA4lacZ3) were found to produce 20 times more infectious viruses than the bulk population, reaching the range of titers obtained with the best TELCeB6-FBdelPASAF clones (Table 3).

Example 9

Assays for transfer of gag-pol or env functions.

To assay for replication-competent viruses, supernatants
5 were used to infect TEL cells (a clone of TE671 cells
harboring an MFGnlslacZ provirus). Infected cells were
passaged for 6 days or longer and their supernatants were
used for infection of fresh TE671 cells. No transmission of
lacZ viruses could be detected (Table 4), demonstrating that
10 the supernatants of pCRIPAMgag--, FBASALF-, FBASAF-, or
FBdelPASAF-transfected TELCeB6 cells were helper-free.
Similar absence of replication competent recombinant
retroviruses was demonstrated using supernatant from a
clone of psiCRIP-MFGnlslacZ cells or from two clones of
15 FLYA-MFGnlslacZ cells (Table 4).

There have been reports that helper-free retroviral vector
stocks may nevertheless contain recombinant retroviruses
(replication incompetent) carrying either gag-pol or env
20 genes (Bestwick et al., Proc Natl Acad Sci USA (1988), 85,
5404-5408, Cosset et al., Virology (1993), 193, 385-395,
Girod et al., Virology (1995), in press). To assay for such
recombinant retroviruses, mobilisation of an MFGnlslacZ
provirus from two indicator cell lines which could cross-
25 complement potential recombinant viruses carrying either
gag-pol or env functional genes was attempted. The TELCeB6
line (Table 2) expressing gag-pol proteins was used as
indicator cell line to test for the presence of env
recombinant (ER) viruses. The TELMOSAF indicator line
30 expressing MoMLV env glycoproteins (obtained by transfection
of FBMOAF, a plasmid expressing the MoMLV env gene using
FBASAF backbone, in TEL cells) was used to detect the
presence of gag-pol recombinant retroviruses (GPR viruses).
After passaging 4-8 days, the supernatants of the infected
35 indicator cells were used to infect either human TE671 cells

or murine NIH3T3 cells.

TELCeB6 cells transfected with various env-expressing constructs, pCRIPAMgag-, FBASAF and FBdelPASAF were 5 compared. Although the supernatants of TELCeB6-FBdelPASAF cells were devoid of replication-competent retroviruses, they were found sporadically to transfer gag-pol genomes (Table 4). No GPR viruses could be detected when less than 2×10^5 virions were used to infect the indicator cells. 10 Similarly TELCeB6 indicator cells infected with various helper-free viruses were shown sporadically to release lacZ virions (Table 4). The number depended both on the env-expression vector used and on the virus input quantity. Compared to lacZ viruses generated using pCRIPAMgag- 15 plasmid, the frequency of detection of the env-recombinant viruses was lower for supernatants generated by using FBASAF and FBdelPASAF constructs (Table 4). For FBdelPASAF construct when less than 5×10^5 MFGnlslacZ(A) helper-free 20 virions were used to infect the indicator cells, no ER retroviruses could be detected. From these experiments, it could be estimated that a supernatant, produced from TELCeB6-FBdelPASAF cells, containing 1×10^7 infectious units 25 of MFGnlslacZ retroviral vector contained no replication-competent virus, and about 100 gag-pol and 100 env recombinant retroviruses.

Table 4. Transfer of packaging function

5	Producer cell	Indicator cell	Input virus ^a (lacZ-i.u.)	Detection ^b		
				++	+	-
Replication competent virus						
	psiCRIP lacZ 5	TEL	2x10 ⁴	0/4	0/4	.4/4
10	TELCeB6-pCRIPAMgag-	TEL	5x10 ⁶	0/4	0/4	4/4
	TELCeB6-FBASAF	TEL	5x10 ⁶	0/4	0/4	4/4
	TELCeB6-FBdelPASAF	TEL	5x10 ⁶	0/4	0/4	4/4
15	FLYA4 lacZ 3	TEL	1x10 ⁷	0/4	0/4	4/4
	FLYA4 lacZ 7	TEL	1x10 ⁷	0/4	0/4	4/4
Gag-pol recombinant						
20	TELCeB6-FBdelPASAF 7	TELMOSAF	2x10 ⁷	0/4	1/4	3/4
	TELCeB6-FBdelPASAF 7	TELMOSAF	2x10 ⁶	0/4	2/4	2/4
	TELCeB6-FBdelPASAF 7	TELMOSAF	2x10 ⁵	0/4	2/4	2/4
	TELCeB6-FBdelPASAF 7	TELMOSAF	2x10 ⁴	0/4	0/4	4/4
Env recombinant						
25	TELCeB6-pCRIPAMgag-	TELCeB6	5x10 ⁶	2/4	1/4	1/4
	TELCeB6-pCRIPAMgag-	TELCeB6	5x10 ⁵	1/4	1/4	2/4
	TELCeB6-pCRIPAMgag-	TELCeB6	5x10 ⁴	0/4	2/4	2/4
30	TELCeB6-FBASAF	TELCeB6	5x10 ⁶	0/4	2/4	2/4
	TELCeB6-FBASAF	TELCeB6	5x10 ⁵	0/4	1/4	3/4
	TELCeB6-FBASAF	TELCeB6	5x10 ⁴	0/4	1/4	3/4
35	TELCeB6-FBdelPASAF	TELCeB6	5x10 ⁶	0/4	1/4	3/4
	TELCeB6-FBdelPASAF	TELCeB6	5x10 ⁵	1/4	3/4	0/4
	TELCeB6-FBdelPASAF	TELCeB6	5x10 ⁴	0/4	0/4	4/4

a: number of lacZ i.u. used to infect indicator cells

b: number of incidence out of four experiments. The ranges of lacZ titers rescued from infected indicator cells are shown for each virus input: >100 lacZ i.u./ml (++) , 1-100 lacZ i.u./ml (+) and <1 lacZ i.u./ml (-).

Titers were determined on TE671 cells for replication competent virus and env recombinant and NIH3T3 cells for

gag-pol recombinant.

Example 10

In order to confirm resistance to complement and absence of replication competent virus in our best packaging lines, MFGnlslacZ(A) and (RD) harvested from FLYA13 and FLYRD18, respectively, after polyclonal transduction of MFGnlslacZ (Table 3 above) were tested for stability in fresh human serum and generation of replication competent virus. Titers of MFGnlslacZ(RD) from FLYRD18 after 1 hr incubation with 3 independent samples of fresh human serum were 80 to 120 % of control incubations, while titers of MFGnlslacZ(A) from FLYA13 were 50 to 90 % of controls (data not shown). No replication competent virus was detected in the same assay described above (Table 4) when 1×10^7 i.u. each of MFGnlslacZ(A) and (RD) were tested.

EXAMPLE 11.

20 Generation of plasmids.

CeB plasmid (Fig. 5) expressing MoMLV gag-pol gene, was further modified to remove the splice donor site located in the leader region. A 272 bp fragment was PCR-generated by using OUSD- (5'-TCTCGCTTCTGTTCGCGCG) and OLSD- (5'-TCGATCAAGCTTGCAGCCGCGGTGGTGGTGGTGGTGGTCC) as primers and further digested with BssHII and HindIII. A 1008 bp HindIII-XhoI fragment isolated from CeB (encompassing a part of leader sequence and beginning MoMLV gag) and the PCR fragment were co-inserted into pCeB from which the 1275 bp BssHII-XhoI fragment (encompassing R-U5-leader-gag) had been removed. The resulting plasmid, named pCeB DS- (Fig. 5), beared the deletion of splice donor (SD) site and a NotI restriction site created just downstream to the lost SD site.

A series of gag-pol expression plasmids in which the MoMLV LTR promoter was replaced by the human cytomegalovirus immediate early promoter (hCMV promoter) was derived from both CeB DS- and hCMV-G (Yee et al., 1994 PNAS, 91: 9564- 9568), a plasmid used as a source for the hCMV promoter. A 5 NotI-filled/EcoRI 7260 bp fragment was isolated from CeB DS- and cloned into hCMV-G which had been opened with SalI (further rendered blunt-ended) and EcoRI to remove the VSV-G gene. The resulting plasmid was cutted with ClaI and EcoRI 10 to remove a 1155 bp fragment encompassing sequence derived from 3'-LTR and SV40 polyA sequence and self-ligated after filling both protruding DNA ends. The resulting plasmid, 15 named phCMV-intron (Fig. 5), had gag-pol and bsr ORFs inserted between the CMV promoter and rabbit beta-globin polyA post-transcriptional regulatory sequences.

An intermediate plasmid was generated by sub-cloning a 7260 bp EcoRI fragment (isolated from CeB DS-) into hCMVG opened with EcoRI. A 1155 bp fragment (encompassing sequence 20 derived from 3'-LTR and SV40 polyA sequence) was removed from this intermediate plasmid which was then re-circularized by self ligation after filling both ends. The resulting plasmid, named phCMV+intron 2P (Fig. 5), was digested with NotI and the vector was treated with klenow 25 enzyme. A 1440 bp fragment (encompassing hCMV promoter and rabbit beta-1 globin intron B (Rohrbaugh et al., 1985 Mol. Cell Biol, 5: 147-160)) was isolated from phCMV+intron 2P by NotI/EcoRI digestion. This fragment was further treated with klenow enzyme and ligated back into the vector. The 30 resulting plasmid, named hCMV+intron (Fig. 5), could express gag-pol and bsr genes driven by the hCMV promoter and beared an intron sequence derived from rabbit beta-1 globin intron B having both SD and SA (splice acceptable) sites.

35 A 2450 bp fragment was removed from phCMV+intron 2P by

NotI/XhoI digestion. The resulting vector fragment was then used to co-ligate a 1330 bp fragment (containing hCMV promoter + 5' end of rabbit beta-1 globin intron B (with SD site)) isolated from phCMVG by ApaI-filled/NotI digestion 5 and a 1 kb fragment isolated from phCMV+intron 2P by NotI-filled/XhoI digestion. Compared to phCMV+intron 2P, the resulting plasmid, named hCMV+SD intron (Fig. 5), had the deletion of the 3' end of the rabbit beta-1 globin intron B and thus no SA site in the leader region.

10

Construct phCMV+leader (Fig. 5) has been described elsewhere (Savard et al., unpublished). This plasmid, in which gag-pol and bsr genes were driven by the hCMV promoter, had the MoMLV SD site in the leader region.

15

Gag-pol expression.

The different constructs, including the parental CeB plasmid, were analysed comparatively in a complementation assay after transfection in TEL-FBdelPASAF cells expressing 20 4070A-MLV (amphotropic) envelope and harboring a MFGnlslacZ provirus. The transient production of lacZ retroviruses as well as the stable production of lacZ retroviral vectors after selection with blasticidin S were determined (Table 5). All the constructs were able to rescue infectious lacZ 25 retroviruses indicating the expression of gag-pol proteins after transient transfection. Most likely due to the efficient hCMV and rabbit beta-1 globin intron B (post)-transcriptional regulatory sequences, hCMV+intron was particularly potent in transient retroviral vector 30 production. However, 10 times less blasticidin-resistant colonies were obtained with hCMV+intron comparatively to CeB, and stable lacZ virus production from hCMV+intron was about 5-10 times lower than that of CeB. Clonal examination of lacZ retrovirus production from blasticidin-resistant 35 colonies indicated that 80-90% of colonies could express

high levels of gag-pol proteins for both hCMV+intron and CeB plasmids. In contrast, despite variation in their ability to form blasticidin-resistant colonies after transfection and despite their ability to express gag-pol proteins from transient transfectants, all other constructs had a weak capacity for rescuing lacZ retroviral vectors from stable transfectants (Table 5).

Table 5. Comparative study of gag-pol-bsr plasmids.

gag-pol-bsr plasmid	Transient (lacZ i.u./ml)	no clones bsr ⁺	Stable (lacZ i.u./ml)	% gag-pol /bsr
Ceb	300/ml	50	10 ⁷	90%
Ceb DS-	144/ml	5	10 ⁵	50%
hCMV+intron 2P	ND	20	10 ⁶	50%
hCMV-intron	812/ml	0	-	-
hCMV+SD intron	150/ml	1000	10 ²	nd
hCMV+leader	328/ml	1000	10 ² -10 ³	nd
hCMV+intron	12000/ml	5	10 ⁶ -10 ⁷	80%

Northern blot analyses were performed on stable transfectants (blasticidin-resistant) obtained with some of the gag-pol-bsr plasmids. As expected, the results (not shown) displayed a correlation between expression of gag-pol mRNAs and gag-pol protein expression detected by rescue analysis (Table 5). CeB construct was found to produce 2-3 fold more gag-pol mRNAs compared to hCMV+intron. Interestingly, an unexpected 2.45 kb RNA band was found for hCMV+intron construct at a ratio of 2:1 compared to the abundancy of the gag-pol mRNA band (at 5.95 kb). Further

investigations by using other probes revealed that a cryptic splice donor (SD) site located in the gag gene (right in the middle of the CA coding region at position 1596-1597 -numbering according to Shinnick et al., 1981 *Nature* (London) 293: 543-548) was activated in this latter construct. The 2.45 RNA species, lacking the 3' half of the gag gene and most of the pol gene, is unlikely to give rise to any useful translational product. It is therefore interesting to notice that hCMV+intron construct was able to give rise to slightly more transcripts (gag-pol 5.95 mRNA + 2.45 alternative RNA band) compared to gag-pol mRNA expressed from CeB construct. Therefore we decided to inactivate the cryptic SD site in the hCMV+intron construct in order to increase the ratio of gag-pol mRNAs.

15

Assays for transfer of gag-pol functions.

Although the supernatants of packaging cell lines generated with CeB gag-pol expression construct were devoid of replication-competent retroviruses, they were found sporadically to transfer gag-pol genomes (example 9, Table 4) (Cosset et al., 1995 *J. Virol.* 69: 7430-7436). Because gag-pol-bsr constructs generated here by using the hCMV promoter had much less retroviral sequences homologous to the retroviral vector than the parental CeB construct (Fig. 5), they are less likely to give rise to gag-pol recombinant (GPR) viruses. Therefore, the most efficient gag-pol-bsr plasmids, hCMV+intron and CeB, were further analysed for emergence of GPR viruses. To assay for such recombinant retroviruses, we attempted to mobilise an lacZ provirus from an indicator cell lines which could cross-complement potential recombinant viruses carrying gag-pol functional genes. Results displayed in Table 6 showed that consistently with data reported previously (example 9, Table 4) (Cosset et al., 1995 *Supra*), lacZ retrovirus vectors generated by using CeB gag-pol construct were contaminated with GPR viruses. In

contrast lacZ retrovirus vectors generated by using hCMV+intron construct were completely devoid of such GPR viruses, suggesting that this construct was improved compared to CeB with respects with emergence of recombinant viruses.

5

Table 6. Comparative study of gag-pol-bsr plasmids.

plasmid	input virus (lacZ i.u.) ^a	no of experiments giving titres of ^b		
CeB	5x10 ⁶	5	3	0
	5x10 ⁵	2	4	2
	5x10 ⁴	0	1	7
hCMV+intron	5x10 ⁶	0	0	8
	5x10 ⁵	0	0	8
	5x10 ⁴	0	0	8

15

4x10E4 cells of TEL/MOSAF in 24 wells were challenged with lacZ(A) of i.u. indicated in the table (a), and incubated at 37°C for 3 days. Cells were trypsinized and transferred into small flasks. Cell sup was harvested on day 5 after lacZ(A) challenge and plated on either TE571 (not shown) and 3T3 cells (b). No lacZ was mobilized into TE671 at all. LacZ(A) from CMV-int 10 again did not rescue lacZ from TEL/MOSAF.

Example 12

25 Generic primers to detect D-type (Medstrand and Blomberg J.Virol. (1993) 67:6778-6787), C-type (Shih et al., J Virol. (1989) 63:64-75), human endogenous virus RTVL-H (Wilkinson et al., J.Virol. (1993) 67:2981-2989), by RT-PCR were employed (Patience et al., *supra*). Primers to detect 30 mouse endogenous VL30 element (Adams et al Mol.Cel.Biol. (1988) 8:2989-2998), and MFGnlslacZ RNA were designed and synthesized (TABLE X). Overnight supernatants (in 4ml of culture medium) from 106 cells of GP+EAM12lacZ25, FLYA4lacZ3

and TELCeB6FBASALF cells (Table 3) were harvested and centrifuged in sucrose gradient as described previously (Patience et al., J.Virol., 70:2654-2657). Fractions containing retrovirus particles were collected, and RNA extracted. One twentieth of the RNA preparation or dilution's thereof were applied to RT-PCR as described previously (Table X). A 1/200 of RNA harvested from GP+EAM12lacZ25 cells was positive for VL30 RNA. MFGnlslacZ RNA was found from 1/20 of RNA from GP+EAM12lacZ and 10 TELCeB6FBASALF cells and 1/200 of RNA from FLYA4lacZ3 cells. The primer combinations for RTVL-H, C- and D-type RNA did not give detectable PCR product.

15 **Table 7. RT-PCR detection of endogenous retrovirus RNA associated with virus particles.**

rt-PCR of virion associated RNA from*					
20	RNA	primer (5'-3') forward(F)/reverse(R)	GP+EAM12 lacZ25	FLYA4 lacZ3	TELCeB6F BASALF
25	MFGnls lacZ	F) CTCTGGCTCACAGTACGACGTAG R) CCATCAATCCGGTAGGTTTCCG	+	++	+
30	C-type	F) CARRGKTTCAARAACWSYCCCAC R) AGYARVGTAGCNGGGTTHAGG	-	-	-
35	D-type	F) TCCCCCTGGAATACTCCTGTTTYGT R) CATTCTTGTGGTAAACTTCCAYTG	-	-	-
	RTVL-H	F) CCTCACCCCTGATCACRYTTG R) GAATTATGTCTGACAGAAAGGG	NT	-	-
	VL30	F) GTTGACATCTGCAGAGAAAGACC R) TCTGAGGTCTGTACACACAATGG	++	NT	NT

a: -, not detected; + detected in 1/20 RNA preparation; ++ detected in 1/200 RNA preparation; NT, not tested because the cells do not possess the corresponding genes.

5

EXAMPLE 13.**Generation of gag-pol pre-packaging cells by using TE671 cells.**

10 CeB, a plasmid designed to over-express MoMLV gag and pol proteins was introduced in TE671 human rhabdomyosarcoma cells (ATCC CRL8805). After selection with blasticidin, 50 bsr-positive colonies were isolated and the RT (reverse transcriptase) activity was analysed in their supernatants.

15 12 TE671-CeB (TECeB) clones with high RT activity were selected for further analysis. The best TECeB clone, clone #15, had a RT activity roughly equivalent to that TELCeB6 cells (Cosset et al., J. Virol. 69:7430-7436 (1995); see also Example 7, Table 6 in this patent application) but

20 displayed 2-3 fold more gag-precursors into cells as demonstrated in immunoblots by using anti-CA antibodies. The biological activity of gag-pol proteins expressed in the six best TECeB clones was further confirmed by their ability to produce infectious retroviruses in a complementation assay.

25 A lacZ provirus was introduced into each of the TECeB clones by polyclonal cross-infection by using lacZ(RD114) helper-free retrovirus vectors. FBMO-SALF, a MoMLV env expression plasmid (Cosset et al., J. Virol. 69:6314-6322), was then transfected in each of the TECeB-lacZ lines and in the

30 TELCeB6 cell line for comparison. After selection with phleomycin, the titer of lacZ retrovirus vectors was determined in the supernatant of pools of phleomycin-resistant colonies for each TECEB-lacZ-FBMO-SALF lines. A

good correlation was found between gag-pol expression into the TE-CeB clones (as determined by RT-assays and anti-gag immunoblots) and their ability to release infectious lacZ particles. TE-CeB15 cells could release approximately the same number of lacZ particles when compared to TELCeB6 cells although TELCeB6 cells had the advantage of being selected for lacZ expression (Cosset et al., J. Virol. 69:7430-7436 (1995)). TE-CeB15 cells were therefore used to derive retroviral packaging cell lines.

10

Construction of env-expression plasmids.

A series of plasmid (Fig. 3) was designed to allow expression of different retroviral envelope genes (isolated from MoMLV, GALV -Gibbon Ape Leukemia Virus-, and MLV-10A1). FBdelPMOSAF (Fig. 3, nucleotide sequence in Fig. 10) and FBdelP10A1SAF, expressing ecotropic MoMLV or MLV-10A1 envelopes, were generated by replacing the BglII/ClaI fragment from FBdelPASAF (Cosset et al., J. Virol. 69:7430-7436 (1995); see also Example 7, Fig. 2 and nucleotide sequence in Fig. 9) encompassing most of the env gene and splice acceptor site with that of MoMLV (position 5407 to 7679, Shinnik et al., 1981) or with that of MLV-10A1 (Ott et al., J. Virol. 64:757-766 (1990)).

Nucleotides 7514-7516 of GALV (Delassus et al., Virology 173:205-213 (1989)) were mutated by PCR-mediated mutagenesis to create a ClaI site (AAG to CGA), thereby introducing a conservative modification (a lysine (amino-acid 665 of GALV env precursor) to an arginine). The BamHI/ClaI fragment (nts 4994 (Delassus et al. Virology 173:205-213 (1989)) to 7517) was then sub-cloned into FBdelPASAF in which the BglII/ClaI encompassing most of the env gene and splice acceptor site had been removed. The resulting plasmid, expressing GALV

envelope glycoproteins, was named FBdelPGASAF (Fig. 3, nucleotide sequence in Fig. 11).

CMV10A1 was generated by inserting a Klenow enzyme-filled EagI/SalI fragment from FBdelP10A1SAF (encompassing 10A1 MLV env gene and phleo selectable marker) into hCMV-G digested with BamHI and filled with Klenow enzyme. The resulting plasmid, CMV10A1 (Fig. 3 and nucleotide sequence in Fig. 13) could express 10A1 envelopes under control of the hCMV promoter and the phleo selectable marker by translation re-initiation.

Generation of a multi-tropic set of TE671-based retroviral packaging lines.

FBdelPRDSAF (Fig. 3, nucleotide sequence in Fig. 12),

15 FBdelPASAF, FBdelPGASAF, FBdelPMOSAF and FBdelP10A1SAF were independently introduced into cells of the TE-CeB15 pre-packaging line, expressing MoMLV gag-pol proteins.

Transfected cells were phleomycin-selected and 15-20 phleo-resistant colonies were isolated for each env-expression 20 plasmid transfected.

Individual colonies were then analysed for expression of envelope glycoproteins by immunoblots on cell lysates by using antibodies against RD114 SU glycoproteins or against Rausher leukemia virus SU (to screen MoMLV, MLV-4070A and

25 MLV-10A1 env-producer clones) or against GALV. The best env-producer colonies as determined in this assay were further analysed by a complementation assay after introducing a lacZ retroviral vector. LacZ pseudotypes released from the different packaging cell lines were titrated by using NIH

30 3T3 cells or TE671 cells as target. Titers higher than 1×10^7 lacZ i.u./ml were obtained for the best clones. Depending on the envelope specificities expressed in these cells, the new

TE671-based retroviral packaging cell lines were named TE-FLYE, TE-FLYA, TE-FLYRD, TE-FLY10A1, and TE-FLYGA and could express the MoMLV, MLV-4070A, RD114, MLV-10A1, and GALV env genes, respectively.

5 Assays for detecting replication-competent retroviruses (RCRs) were performed in the supernatants of these cells and were negative (less than 1/ml).

10 TE671 cells are very potent for transient expression resulting in more than 95% of cells expressing transgene three days after plasmid transfection (Hatzioannou and Cosset, unpublished data, (1996)). The ability of retroviral packaging cell lines to transiently produce retroviral vectors is of crucial importance for gene therapy where 15 vectors carrying toxic gene have to be prepared. Transient expression of retroviral vectors was comparatively determined from cells of the TE-FLYA line and from the BING line (Pear et al., Proc Natl Acad Sci U S A 90, 8392-6 (1993)), a retroviral packaging cell line designed to 20 transiently express retroviral vectors. Results (Table 8) showed that TE-FLYA cells were more efficient for transient expression of a lacZ retroviral vector hence resulting in higher titers.

25 **Table 8. Comparative study of transient production of lacZ vectors.**

packaging cell line	cell number ^a	% transfected cells ^b	transient titer ^c
BING	281	5.3	2x10 ²
TE-FLYA	117	35	1.3x10 ³

30 Cells were transfected by MFGnlslacZ retroviral vectors with calcium phosphate precipitation method and titers of lacZ vectors (c) released in cell

supernatant were determined as lacZ i.u./ml at day 3 following transfection. The relative number of cells (a) (average per microscope field) and the % of transfected cells (b) determined after X-gal staining are shown.

5 Retroviral vectors prepared from TE671-based packaging cell lines were analysed for their sensitivity to human-complement mediated inactivation. Experiments were conducted as previously described (Cosset et al., J. Virol. 69:7430-7436 (1995); see also Example 10 in this patent application)
10 by using three human sera of individual donors (Table 9). As expected MLV-A prepared from mouse 3T3 cells were highly sensitive to inactivation after 1 hr incubation with sera. In contrast, titers of lacZ vectors produced from TE-FLYRD cells were 17 to 55% of control incubations, while titers of
15 lacZ vectors from TE-FLYA cells were 1 to 30% of controls.

Table 9. Human serum sensitivity of viruses produced from TE671-based packaging cell lines.

Virus from:	hu56 ^a	hu57 ^a	BTS ^a
3T3/A	<0.2, <0.2	<0.2, <0.2	<0.2, <0.2
TE-FLYE	15, 7.8	16, 11	48, 60
TE-FLYA	1, 0.6	2.2, 7.1	28, 19
TE-FLYRD	17, 22	30, 44	54, 63

20 25 Three human fresh serum samples were tested in duplicate; hu56 (A+), hu57(AB+), BTS(AB+). (a) % control (average for FCS and opti-MEM treatment) is shown.

CLAIMS:

1. A recombinant expression vector comprising a gene of interest and a selectable marker gene, wherein the selectable marker gene is arranged downstream of the gene of interest and a stop codon associated with the gene of interest is spaced from a start codon of said selectable marker gene at a distance which is sufficient to ensure that said selectable marker protein is expressed from the corresponding mRNA as a result of translation reinitiation.
2. A recombinant expression vector according to claim 1 wherein the vector is a viral vector.
3. A recombinant expression vector according to claim 2 wherein the vector is a retroviral vector.
4. A recombinant expression vector according to any one of claims 1 to 3 wherein the gene of interest is included as part of a viral packaging construct.
5. A recombinant expression vector according to any one of the preceding claims wherein the number of nucleotides in the space between the stop codon of the gene of interest and the start codon of the selectable marker is in the range of from 20 to 200 nucleotides.
6. A recombinant expression vector according to claim 5 wherein the number of nucleotides in the space between the stop codon of the gene of interest and the start codon of the selectable marker is in the range of from 60 to 80 nucleotides.
7. A process for producing a cell line in which a gene of interest is expressed, which process comprises:
transforming host cells with an expression vector

according to any one of the claims 1 to 6; and
selectable those cells where expression of the
selection marker gene may be detected.

8. A process according to claim 7 wherein the host cell is a eukaryotic cell.
9. A host cell transformed with a recombinant expression vector according to any one of the claims 1 to 6.
10. A retroviral packaging cell line comprising a host cell transformed with a first and a second recombinant expression vector, said first recombinant expression vector having a packaging-deficient construct comprising a viral gag-pol gene and a first selectable marker gene downstream thereof, and said second recombinant expression vector having a packaging-deficient construct comprising a viral env gene and a second selectable marker gene downstream thereof; wherein the start codon of the first and second selectable markers are spaced from the stop codons of the viral gag-pol gene and the viral env gene respectively by a distance which ensures that said selectable marker protein is expressed from the corresponding mRNA as a result of translation reinitiation.
11. A retroviral packaging cell line according to claim 10 wherein the first selectable marker is a bsr selectable marker and the second selectable marker is a phleo selectable marker.
12. A retroviral packaging cell line according to any one of claims 10 or 11 wherein the packaging-deficient construct comprising the viral gag-pol gene and first selectable marker is the CeB (SEQ ID No 2) expression construct.

13. A retroviral packaging cell line according to any one of claims 10 or 11 wherein the packaging-deficient construct comprising the viral env gene and second selectable marker is the FBdelPASAF (SEQ ID No 5), the FBdelPMOSAF (SEQ ID No 6), the FbdelPGASAF (SEQ ID No 7), the FbdelPRDSAF (SEQ ID No 8), the FbdelPXSAF (Fig. 3), the FbdelP10A1SAF (Fig. 3), or the FBdelPVSVGSAF (Fig. 3) expression construct.
14. A retroviral packaging cell line according to any one of claims 10 or 11 wherein the recombinant expression vector is a packaging-deficient retroviral helper construct.
15. A retroviral packaging cell line according to claim 14 wherein the overlapping sequences between the genomes of the retroviral vector and the packaging-deficient construct is reduced by minimizing the extent of non-coding retroviral sequences in the packaging-deficient genome.
16. A retroviral packaging cell line according to any one of claims 10 to 15 wherein the viral gag-pol gene and the selectable marker are expressed under the control of a non-retroviral promoter.
17. A retroviral packaging cell line according to claim 16 wherein the promoter is fused to rabbit beta-1 globin intron.
18. A retroviral packaging cell line according to claim 16 or claim 17 wherein the promoter is a hCMV promoter.
19. A retroviral packaging cell line according to any one of claims 16 to claim 18 wherein the viral gag-pol gene and the selectable marker is a hCMV+intron (SEQ

ID No3) or a hCMV+intronkaSD (SEQ ID No 4) expression construct.

20. A retroviral packaging cell line according to anyone of claims 10 to 15 wherein the viral env gene and the selectable marker are under the control of a non-retroviral promoter.
21. A retroviral packaging cell line according to claim 20 wherein the promoter is fused to rabbit beta-1 globin intron.
22. A retroviral packaging cell line according to claim 20 or claim 21 wherein the promoter is a hCMV promoter.
23. A retroviral packaging cell line according any one of claims 20 to 22 wherein the viral env gene and the selectable marker is a CMV10A1 (SEQ ID No 9) expression construct.
24. A retroviral packaging cell line according to any one of claims 10 to 23 wherein the cell line is the HT1080 line, the TE671 line, the 3T3 line, the 293 line or the MV-1-LU line.
25. A retroviral packaging cell line according to anyone of claims 10 to 24 wherein the retroviral packaging cells comprises human HT1080 cells and express RD114 envelopes.
26. A retroviral packaging cell line according to anyone of claims 10 to 24 wherein the retroviral packaging cells comprises human TE671 cells and express RD114 envelopes.

27. A process for producing a retroviral packaging cell line in which a gene of interest is expressed, which process comprises:

transforming host cells with a first and a second recombinant expression vector, said first recombinant expression vector having a packaging-deficient construct comprising a viral gag-pol gene and a first selectable marker gene downstream thereof, and said second recombinant expression vector having a packaging-deficient construct comprising a viral env gene and a second selectable marker gene downstream thereof; wherein the start codon of the first and second selectable markers are spaced from the stop codons of the viral gag-pol gene and the viral env gene respectively by a distance which ensures that said selectable marker protein is expressed from the corresponding mRNA as a result of translation reinitiation; and

selecting transformed cells which express said first and/or second marker genes.

28. A packaging deficient construct for use in a process according to claim 27, which expresses a viral gag-pol gene and a selectable marker wherein a start codon of the selectable marker is spaced from a stop codon of the viral gag-pol gene by a distance which ensures that said selectable marker protein is expressed from the corresponding mRNA as a result of translation reinitiation.

29. A packaging deficient construct for use in a process according to claim 27, which expresses a viral env gene and a selectable marker gene; wherein a start codon of the selectable marker is spaced from a stop codon of the viral env gene by a distance which ensures that said selectable marker protein is

expressed from the corresponding mRNA as a result of translation reinitiation.

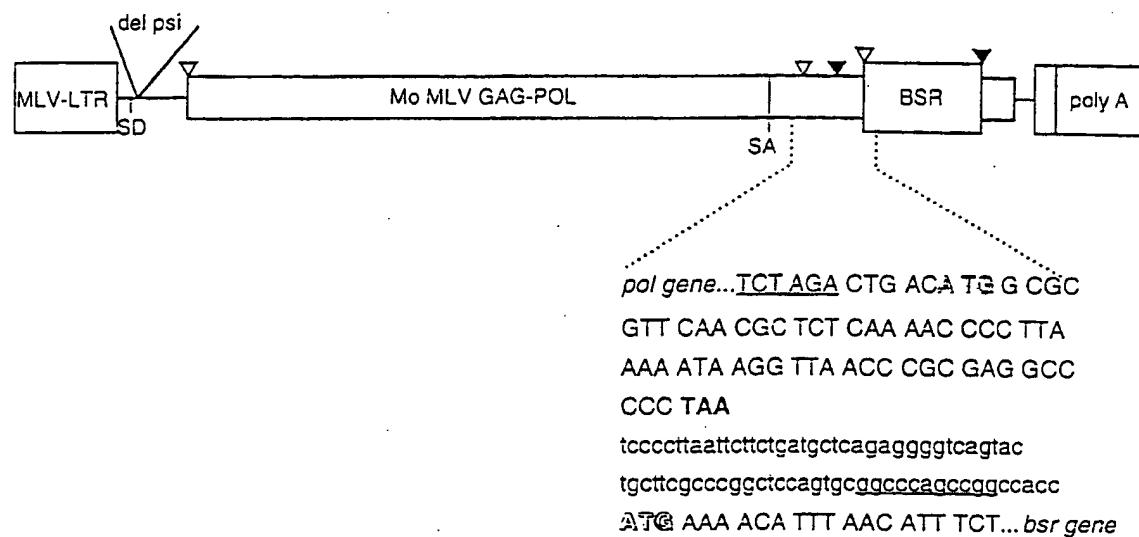


Figure 1. Schematic structure of CeB expression vector

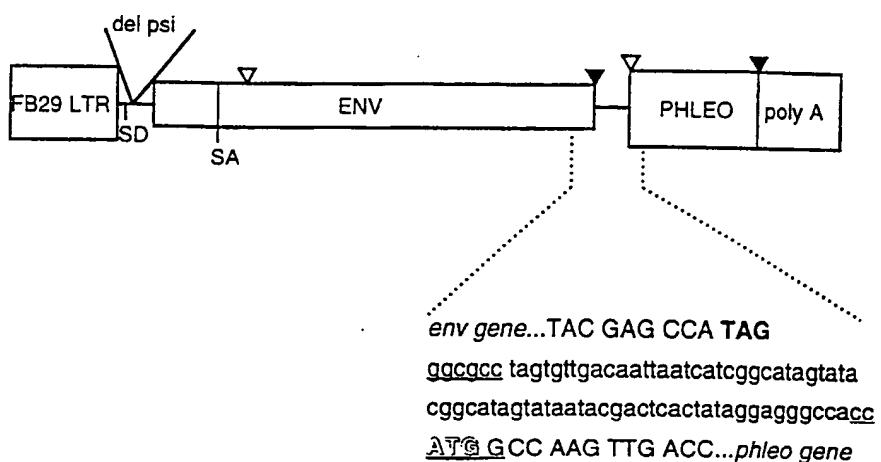


Figure 2. Schematic structure of FBdelPASF expression vector

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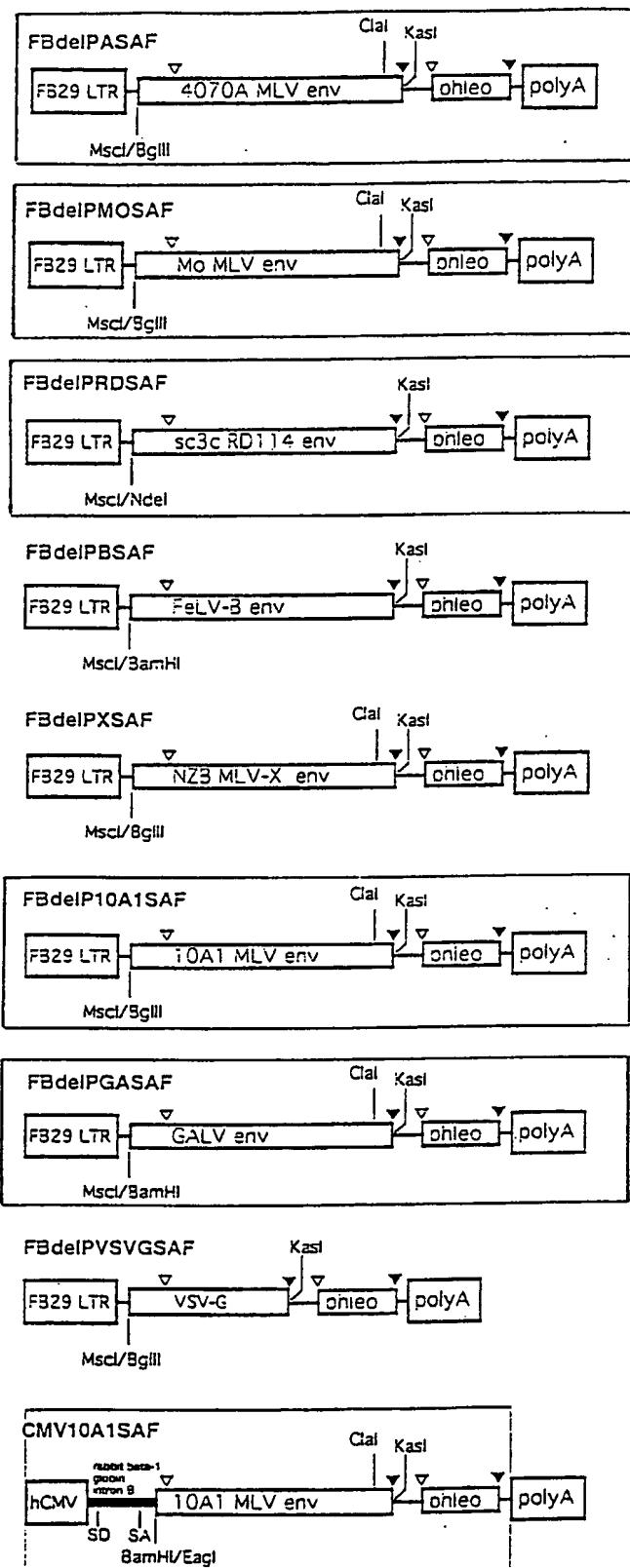


Figure 3. Schematic structure of env expression vectors
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NGAGCTCAGGACAGGTAGAAAGAATGAATAGAACATAAAAGAGACCCTTACTAAATTGA 60
 CCTTAGAGACTGGCTTAAAGATTGGAGACGCCCTCTATCTCTGGCTTGTAAAGAGCCA 120
 GAAATACGCCAACCGTTTCGGCTCACCCATATGAAATCCTTATGGGGACCCCCCCC 180
 CTTTGTCAACCTGCTCAATTCTCTCCCCCTCGATCTAAGACTGATTACAAGCCC 240
 GACTAAAAGGGCTGCAAGCGTGCAGGCCAAATCTGGACACCCCTGGCGAATTGTACC 300
 GGCCAGGACATCCACAAACTAGCCACCCATTCAAGGTGGGAGACTCCGTGTACGTCCGGC 360
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 CCCCACCCTAGCCGGAAAGTCCCCAGCCGCTACGCAACCCGGGCCCCGAGTTGCATCAGC 2460
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Fig. 4

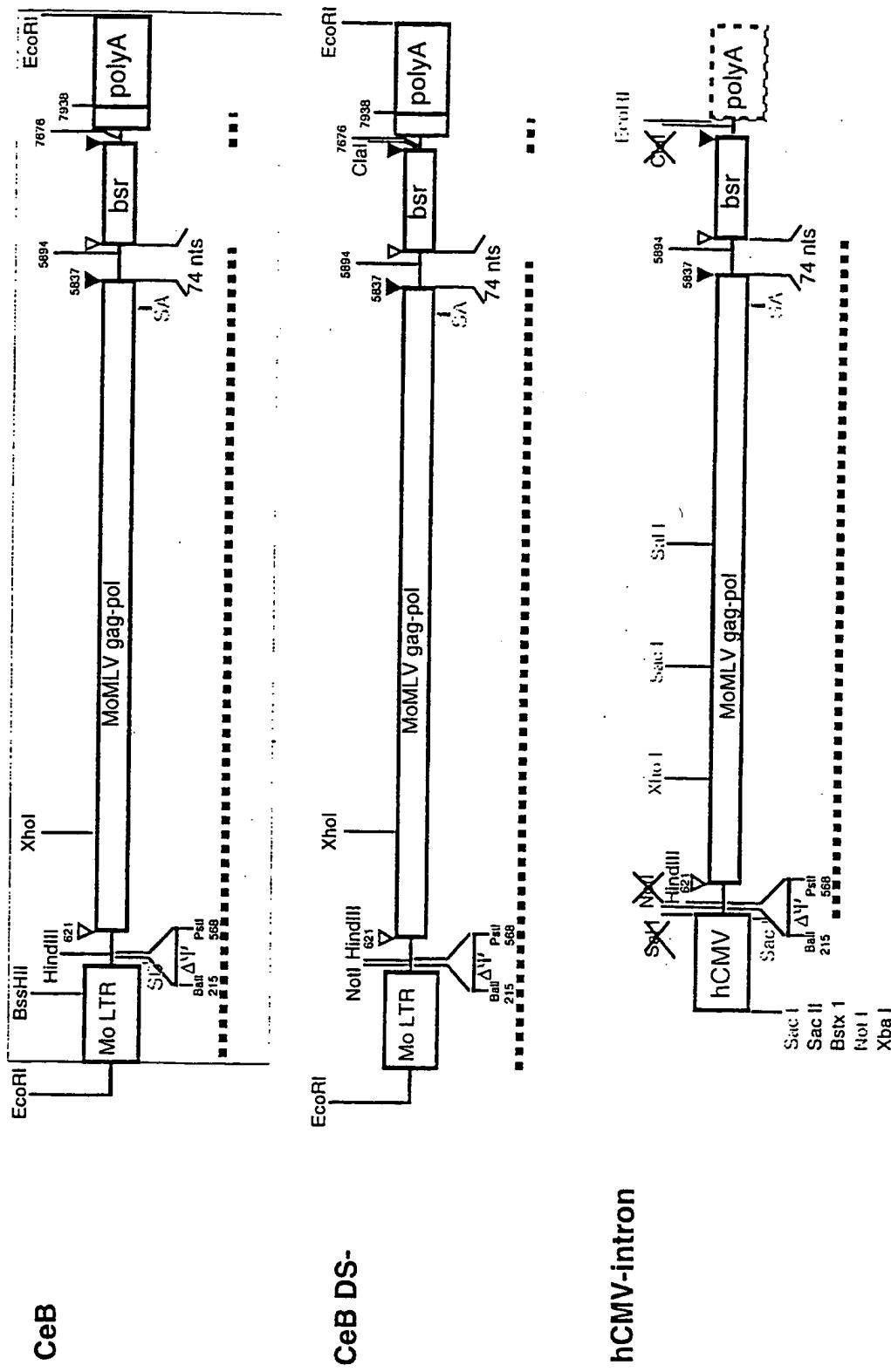


Figure 5. Genetic structure of gag-pol constructs (page 1/3)

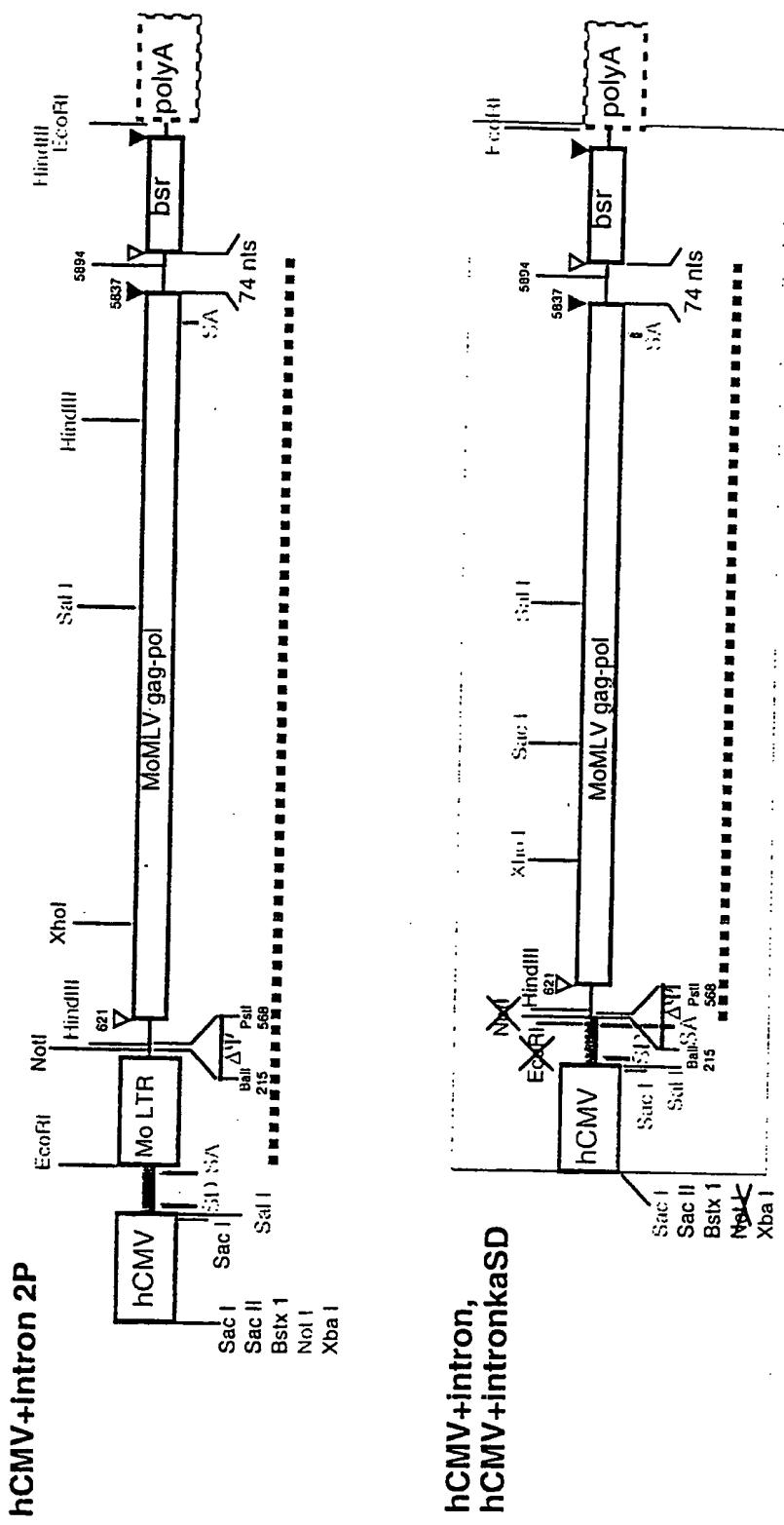


Figure 5. Genetic structure of gag-pol constructs (page 2/3)

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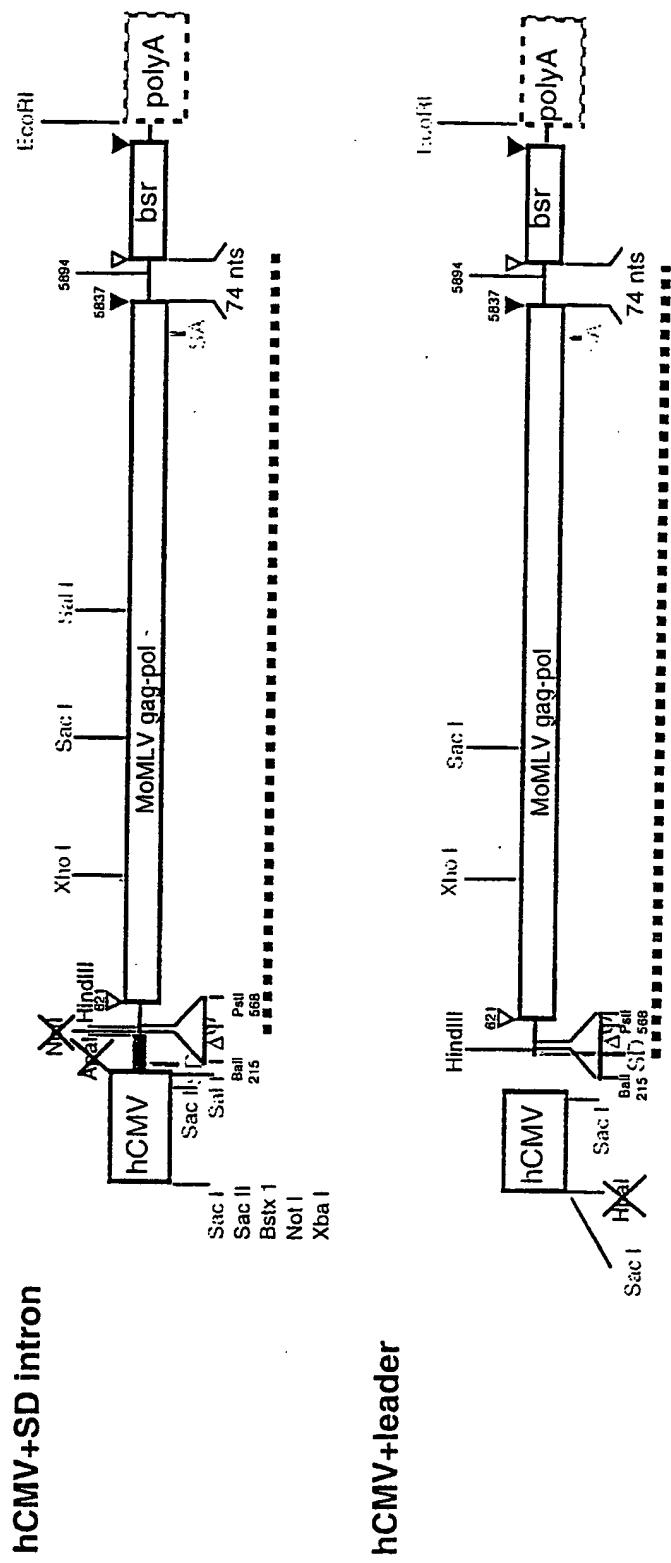


Figure 5. Genetic structure of gag-pol constructs (page 3/3)

Figure 6. CeB Sequence

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AATGAAAAGAC	CCCACCTGTA	GGTTTGGCAA	GCTAGCTTAA	GTAACGCCAT	TTTGCAAGGC	60
ATGGAAAAAT	ACATAACTGA	GAATAGAGAA	GTTCAGATCA	AGGTCAAGGAA	CAGATGGAAC	120
AGCTGAATAT	GGGCCAAACA	GGATATCTGT	GGTAAGCAGT	TCCCTGCCCG	GCTCAGGGCC	180
AAGAACAGAT	GGAACAGCTG	AATATGGGCC	AAACAGGATA	TCTGTGGTAA	GCAGTTCTG	240
CCCCGGCTCA	GGGCCAAGAA	CAGATGGTCC	CCAGATGCGG	TCCAGCCCTC	AGCAGTTCT	300
AGAGAACCAT	CAGATGTTTC	CAGGGTCCC	CAAGGACCTG	AAATGACCT	GTGCCTTATT	360
TGAACTAAC	AATCAGTTCG	CTTCTCGCTT	CTGTTCGCGC	GCTTCTGCTC	CCCGAGCTA	420
ATAAAAGAGC	CCACAACCCC	TCACTCGGGG	CGCAGTCCT	CCGATTGACT	GAGTCGCCG	480
GGTACCCGTG	TATCCAATAA	ACCCCTTTC	AGITGCATCC	GACTGTGTT	CTCGCTGTT	540
CTTGGGAGGG	TCTCCTCTGA	GTGATTGACT	ACCCGTCAGC	GGGGGTCTT	CATTGGGGG	600
CTCGTCCGGG	ATCGGGAGAC	CCCTGCCCC	GGACACCACG	CCCCACCCCG	GGAGGTAAGC	660
TGGAAGCTTC	TGCAAGCATCG	TTCTGTGTTG	TCTCTGTCTG	ACTGTGTTTC	TGTATTGTC	720
TGAGAATATG	GGCCAGACTG	TTACCACTCC	CTTAAGTTTG	ACCTTAGGTC	ACTGAAAGA	780
TGTCGAGCGG	ATCGCTCACA	ACCAAGTCGGT	AGATGTCAAG	AAGAGACGTT	GGGTTACCTT	840
CTGCTCTGCA	GAATGGCCAA	CCTTTAACGT	CGGATGGCCG	CGAGACGGCA	CCTTTAACCG	900
AGACCTCATC	ACCCAGGTTA	AGATCAAGGT	CTTTTCACCT	GGCCCGCATG	GACACCCAGA	960
CCAGGTCCCC	TACATCGTGA	CCTGGGAAGC	CTTGGCTTTT	GACCCCCCTC	CCTGGTCAA	1020
GCCCTTGTGA	CACCCCTAACG	CTCCGCCCTC	TCTCTCTTCA	TCCGGCCCTG	CTCTCCCCCT	1080
TGAACTCTCT	CGTTGACCC	CGCCTCGATC	CTCCTTTTAT	CCAGCCCTCA	CTCCTTCTCT	1140
AGGCGCCAAA	CCTAAACCTC	AAAGTCTTT	TGACAGTGGG	GGGCGCTCA	TCGACCTACT	1200
TACAGAAGAC	CCCCCGCCTT	ATAGGGACCC	AAGACCAAC	CCTTCCGACA	GGGACGGAAA	1260
TGGTGGAGAA	CGGACCCCTG	CGGGAGAGGC	ACCGGACCCC	TCCCCAATGG	CATCTGCCT	1320
ACGTGGGAGA	CGGGAGGCC	CTGTGGCCGA	CTCCACTACC	TCGCAGGCAT	TCCCCCTCCG	1380
CGCAGGAGGA	AACGGGAGAC	TTCAATACTG	GCCGTTCTCC	TCTCTGACC	TTTACAACTG	1440
GAAAAATAAT	AACCCCTTCTT	TTCTGAGAAGA	TCCAGGTTAA	TCGACAGCTC	TGATCGAGTC	1500
TGTTCTCATC	ACCCATCAGC	CCACCTGGGA	CGACTGTCAG	CAGCTGTTGG	GGACTCTGCT	1560
GACCGGAGAA	GAAAAAACAA	GGGTGCTTT	AGAGGCTAGA	AAGGCGGTG	GGGGCGATGA	1620
TGGGCGCCCC	ACTCAACTGC	CCAATGAAGT	CGATGCCGT	TTTCCCCCTG	AGCGCCCGA	1680
CTGGGATTAC	ACCACCCAGG	CAGGTAGGAA	CCACCTAGTC	CACTATGCC	AGTTGCTCCT	1740
AGCGGGCTC	CAAAACGCGG	GCAGAAGCCC	CACCAATTG	GCCAAGGTA	AAGGAATAAC	1800
ACAAGGGCCC	AATGAGTCTC	CCTCGGCTT	CCTAGAGAGA	CTTAAGGAAG	CCTATCGCAG	1860
GTACACTCCT	TATGACCCCTG	AGGACCCAGG	GCAAAAGAA	AATGTCATCA	TGTCCTTCAT	1920
TTGGCAGTCT	GCCCCAGACA	TTGGGAGAAA	GTAGAGAGG	TTAGAGATT	AAAAAAACAA	1980
GACGCTTGA	GATTTGGTTA	GAGAGGCAGA	AAAGATCTTT	AATAAACGAG	AAACCCCGGA	2040
AGAAAGAGAG	GAACGTATCA	GGAGAGAAAC	AGAGGAAAAA	GAAGAACGCC	GTAGGACAGA	2100
GGATGAGCAG	AAAGAGAAAG	AAAGAGATCG	TAGGAGACAT	AGAGAGATGA	GCAAGCTATT	2160
GGCCACTGTC	GTAGTGGAC	AGAAACAGGA	TAGACAGGG	GGAGAACGAA	GGAGGTCCTA	2220
ACTCGATCGC	GACCACTGTC	CCTACTGCAA	AGAAAAGGG	CACTGGGCTA	AGAGTTGTCC	2280
CAAGAAACCA	CGAGGACCTC	GGGGACCAAG	ACCCCGACCC	TCCCTCCCTGA	CCCTAGATGA	2340
CTAGGGAGGT	CAGGGTCAGG	AGCCCCCCC	TGAACCCAGG	ATAACCTCA	AAAGTCGGGG	2400
GCAACCCGTC	ACCTTCCCTGG	TAGATACTGG	GGCCCAACAC	TCCGTGCTGA	CCCAAATCC	2460
TGGACCCCTA	AGTGATAAGT	CTGCGCTGGT	CCAAGGGCT	ACTGGAGGAA	AGCGGTATCG	2520
CTGGACCACG	GATGCAAAG	TACATCTAGC	TACCGGTAAG	GTCACCCACT	CTTTCTCTCA	2580
TGTCACAGAC	TGTCCTATC	CTCTGTTAGG	AAGAGATTG	CTGACTAAC	AAAAGCCCA	2640
AATCCACTTT	GAGGGATCAG	GAGCTCAGGT	TATGGGACCA	ATGGGGCAGC	CCCTGCAAGT	2700
GTTGACCCCTA	AAATAGAGAAG	ATGAGCATCG	GCTACATGAG	ACCTCAAAG	AGCCAGATGT	2760
TTCCTCTAGGG	TCCACATGGC	TGTCTGATTT	TCCTCAGGCC	TGGGCGAAA	CGGGGGGCAT	2820
GGGACTGGCA	GTCGCCAAG	CTCCCTCTGAT	CATAACCTCTG	AAAGCAACCT	CTACCCCCGT	2880
GTCCATAAAA	CAATACCCCA	TGTCACAAGA	AGCCAGACTG	GGGATCAAGC	CCCACATACA	2940
GAGACTGTG	GACCAGGGAA	TACTGGTACC	CTGCCAGTCC	CCCTGGAACA	CGCCCCCTGCT	3000
ACCCGTTAAC	AAACCAGGG	CTAATGATTA	TAGGCCGTG	CAGGATCTGA	GAGAAGTCAA	3060
CAAGGGGTG	GAAGACATCC	ACCCCAACCGT	GCCCAACCC	TACAACCTCT	TGAGCGGGCT	3120
CCCACCGTC	CACCACTGGT	ACACTGTGCT	TGATTTAAAG	GATGCCCTTT	TCTGCTGAG	3180
ACTCCACCCC	ACCAAGTCAGC	CTCTCTTCG	CTTCTGAGTGG	AGAGATCCAG	AGATGGAAT	3240
CTCAGGACAA	TTGACCTGGA	CCAGACTCCC	ACAGGGTTTC	AAAAACAGTC	CCACCCCTGTT	3300
TGATGAGGCA	CTGCACAGAG	ACCTAGCAGA	CTTCCGGATC	CAGCACCCAG	ACTTGATCCT	3360
GCTACAGTAC	GTGGATGACT	TACTGCTGGC	CGCCACTTCT	GAGCTAGACT	GCCAACAAGG	3420
TACTCGGGCC	CTGTTACAAA	CCCTAGGGAA	CCTCGGGTAT	CGGGCCTCG	CCAAGAAAAGC	3480
CCAAATTGTC	CAGAAACAGG	TCAAGTATCT	GGGGTATCTT	CTAAAAGAGG	GTCAGAGATG	3540
GCTGACTGAG	GCCAGAAAAG	AGACTGTGAT	GGGGCAGCCT	ACTCCGAAGA	CCCCCTCGAC	3600
ACTAAAGGGAG	TTCCTAGGG	CGGCAGGCTT	CTGTCGCTC	TGGATCCCTG	GGTTTGCAGA	3660
AATGCCAGCC	CCCTTGTACC	CTCTCACCAA	AACGGGACT	CTGTTTAATT	GGGGCCCAGA	3720
CCAACAAAAG	GCCTATCAAG	AAATCAAGCA	AGCTCTTCTA	ACTGCCAG	CCCTGGGGTT	3780
GCCAGATTG	ACTAAGCCCT	TTGAACCTT	TGTCGACGAG	AAGCAGGCT	ACGCCAAAGG	3840
TGTCTTAACG	CAAAACTGG	GACCTTGGCG	TCGGCCGGTG	GCCTACCTGT	CCAAAAGCT	3900
AGACCCAGTA	GCAGCTGGGT	GGCCCCCTTG	CCTACGGATG	GTAGCAGCCA	TTGCGCTACT	3960
GACAAAGGAT	GCAGGCAAGC	TAACCATGGG	ACAGCCACTA	GTCATTCTGG	CCCCCATGC	4020
AGTAGAGGCA	CTAGTCAAAC	AACCCCCCGA	CCGCTGGCTT	TCCAACGCC	GGATGACTCA	4080

Figure 6. CeB Sequence

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CTATCAGGCC	TTGCTTTG	ACACGGACCG	GGTCCAGTTC	GGACCGGTGG	TAGCCCTGAA	4140
CCCGGCTACG	CTGCTCCAC	TGCCTGAGGA	AGGGCTGCAA	CACAAGTGC	TTGATATCCT	4200
GGCCGAAGCC	CACGGAAACCC	GACCCGACCT	AACGGACCA	CCGCTCCAG	ACGCCGACCA	4260
CACCTGGTAC	ACGGATGGAA	GCAGTCTCTT	ACAAGAGGG	CAGCGTAAGG	CGGGAGCTGC	4320
GGTGACCA	GAGACCGAGG	TAATCTGGC	TAAGCCCTG	CCAGCCGGGA	CATCCGCTCA	4380
GCGGGCTGAA	CTGATAGCAC	TCACCCAGGC	CCTAAAGATG	GCAGAAGGTA	AGAAGCTAAA	4440
TGTTTATACT	GATAGCGTT	ATGCTTTGC	TACTGCCCAT	ATCCATGGAG	AAATATACAG	4500
AAGGGCTGGG	TTGCTCACAT	CAGAAGGCAA	AGAGATCAA	AATAAAGACG	AGATCTTGGC	4560
CCTACTAAA	GCCCTCTTC	TGCCCCAAAG	ACTTAGCATA	ATCCATTGTC	CAGGACATCA	4620
AAAGGGACAC	AGCGCCGAGG	CTAGAGGCAA	CCGGATGGCT	GACCAAGCGG	CCCGAAAGGC	4680
AGCCATCACA	GAGACTCCAG	ACACCTCTAC	CCTCCTCATA	AAAATTCTAT	CACCCCTACAC	4740
CTCAGAACAT	TTTCATTACA	CAGTGACTGA	TATAAAGGAC	CTAACCAAGT	TGGGGGCCAT	4800
TTATGATAAA	ACAAAGAAGT	ATTGGGTCTA	CCAAGGAAAA	CCTGTGATGC	CTGACCAGTT	4860
TACTTTGAA	TTATTAGACT	TTCTTCATCA	GCTGACTCAC	CTCAGCTTCT	AAAAAATGAA	4920
GGCTCTCCTA	GAGAGAAAGCC	ACAGTCCCTA	CTACATGCTG	AACCGGGATC	AAACACTCAA	4980
AAATATCACT	GAGACCTGCA	AAGCTTGTC	ACAAGTCAAC	GCCAGCAAGT	CTGCCGTTAA	5040
ACAGGGAAC	AGGGTCCGCG	GGCATCGGCC	CGGCACTCAT	TGGGAGATCG	ATTTCACCGA	5100
GATAAAGCCC	GGATTGTATG	GCTATAAATA	TCTCTAGTT	TTTATAGATA	CCTTTCTGG	5160
CTGGATAGAA	GCCCTCCCAA	CCAAGAAGA	AACCGCCAAG	GTCGTAACCA	AGAAGCTACT	5220
AGAGGAGATC	TTCCCCAGGT	TGGCGATGCC	TCAGGTATTG	GGAACTGACA	ATGGGCCTGC	5280
CTTCGTCTCC	AAAGGTGAGTC	AGACAGTGGC	CGATCTGTTG	GGGATTGATT	GGAAATTACA	5340
TTGTGCATAC	AGACCCCAA	GCTCAGGCCA	GGTAGAAAGA	ATGAATAGAA	CCATCAAGGA	5400
GACTTTAACT	AAATTAAACGC	TTGCAACTGG	CTCTAGAGAC	TGGGTCTCC	TACTCCCCTT	5460
AGCCCTGTAC	CGAGCCCGCA	ACACGCCGGG	CCCCCATGGC	CTCACCCCAT	ATGAGATCTT	5520
ATATGGGCA	CCCCCGCCCC	TTGTAACATT	CCCTGACCCCT	GACATGACAA	GAGTTACTAA	5580
CAGCCCCCT	CTCCAAGCTC	ACTTACAGGC	TCTCTACTTA	GTCCAGCACG	AAGTCTGGAG	5640
ACCTCTGGCG	GCAGCCTACC	AAGAACAACT	GGACCGACCG	GTGGTACCTC	ACCCTTACCG	5700
AGTCGGCAGC	ACAGTGTGG	TCCGCCGACA	CCAGACTAAG	AACCTAGAA	CTCGCTGGAA	5760
AGGACCTTAC	ACAGTCTGC	TGACCACCCC	CACCGCCCTC	AAAGTAGACG	GCATCGCAGC	5820
TTGGATACAC	GCCGCCACCG	TGAAGGCTGC	CGACCCCGGG	GGTGGACCAT	CCTCTAGACT	5880
GACATGGCGC	GTTCAACGCT	CTCAAAACCC	CTTAAAATA	AGGTTAACCC	GCGAGGCC	5940
CTAATCCCC	TAATTCTTCT	GATGCTCAGA	GGGGTCAGTA	CTGCTTCGCC	CGGCTCCAGT	6000
GCGGCCAGC	CGGCCACCAT	GAAAACATT	AAACATTCTC	AACAAGATCT	AGAATTAGTA	6060
GAAGTAGCGA	CACAGAAAGAT	TACAATGCTT	TATGAGGATA	ATAAACATCA	TGTGGGAGCG	6120
GCAATTGCTA	CGAAAACAGG	AGAAATCATT	TCGGCAGTAC	ATATTGAGC	GTATATAGGA	6180
CGAGTAAC	TTTGTGCAGA	AGCCATTGCG	ATTGGTAGTG	CAGTTGCGA	TGGACAAAAG	6240
GATTTGACA	CGATTGTAGC	TGTTAGACAC	CCTTATTCTG	ACGAAGTAGA	TAGAAGTATT	6300
CGAGTGGTAA	GTCCCTGTGG	TATGTGTAGG	GAGTTGATT	CAGACTATGC	ACCAGATTGT	6360
TTTGTGTTAA	TAGAAATGAA	TGGCAAGTTA	GTCAAAACTA	CGATTGAGA	ACTCATTCCA	6420
CTCAAATA	CCCGAAATTA	AAAGTTTAC	CACCAAGCTT	ATCGATTAGT	CCAATTGTTGTT	6480
AAAGACAGGA	TATCAGTGGT	CCAGGCTCTA	GTTTTGACTC	AACAATATCA	CCAGCTGAAG	6540
CCTATAGAGT	ACGAGCCATA	GATAAAATAA	AAAGTTTAT	TTAGTCTCCA	AAAAAAAGGGG	6600
GGAATGAAAG	ACCCACCTG	TAGGTTGGC	AAGCTAGCTT	AAGTAACGCC	ATTITGCAAG	6660
GCATGGAAAA	ATACATAACT	GAGAATAGAG	AAGTTCAGAT	CAAGGTCAGG	AACAGATGGA	6720
ACAGTCGAGA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA	AATAAAGCAA	TAGCATCACA	6780
AATTTACAAA	ATAAACGCTT	TTTTTCACTG	CATTCTAGTT	GTGGTTGTC	CAAACTCATC	6840
AATGTATCTT	ATCATGTC	GATCCCCAGG	AAGCTCTCT	GTGTCCTCAT	AAACCTAAC	6900
CTCCTCTACT	TGAGGAGACA	TTCCAATCAT	AGGTGCCCCA	TCCACCCCT	GTGTCCTCCT	6960
TTAAATTAGG	TCACTTAACA	AAAAGGAAAT	TGGGTAGGGG	TTTTTCACAG	ACCGCTTTCT	7020
AAGGGTAATT	TTAAATATC	TGGGAAGTCC	CTTCCACTGC	TGTGTTCCAG	AAGTGTGGT	7080
AAACAGCCCA	CAAATGTCAA	CAGCAGAAC	ATACAAGCTG	TCAGTTTGC	ACAAGGCC	7140
ACACCCCTGC	TCATCAAGAA	GCACGTGGT	TGCTGTGTTA	GTAATGTGCA	AAACAGGAGG	7200
CACATTTC	CCACCTGTGT	AGGTTCCAAA	ATATCTAGTG	TTTTCATTT	TACTTGGATC	7260
AGGAACCCAG	CACTCCACTG	GATAAGCATT	ATCCCTTATCC	AAAACAGCT	TGTGGTCAGT	7320
GTTCATCTGC	TGACTGTCAA	CTGTAGCATT	TTTTGGGGTT	ACAGTTGAG	CAGGATATT	7380
GGTCCTGTAG	TTTGCTAAC	CACCTGCGAG	CTCCAAAGGT	TCCCCACCAA	CAGCAAAAAAA	7440
ATGAAAATT	GACCCCTGAA	TGGGTTTCC	AGCACCATT	TCATGAGTT	TTTGTGTC	7500
TGAATGCAAG	TTAACACATAG	CAGTACCC	AATAACCTCA	GTTTTAACAG	TAACAGCTTC	7560
CCACATCAAA	ATATTTCAC	AGGTTAAGTC	CTCATTTAAA	TTAGGCAAAG	GAATT	7616

Figure 7. hCMV+intron Sequence

AGATCTCCCG	ATCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	60
AGCCAGTATC	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGCAC	GAGCAAAATT	120
TAAGCTACAA	CAAGGCAAGG	CTTGACCGAC	AATTGATGAA	AGAATCTGCT	TAGGGTTAGG	180
CGTTTGCGC	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	240
AGTTATTAAT	AGTAATCAAT	TACGGGGTCA	TTAGTTCATC	GCCCCATATAT	GGAGGTTCCGC	300
GTTACATAAC	TTACGGTAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCATTG	360
ACGTCAATAA	TGACGTATGT	TCCCATAGTA	ACGCAATAG	GGACTTTCCA	TTGACGTCAA	420
TGGGTGGACT	ATTTACCGTA	AACTGCCAC	TTGGCAGTAC	ATCAAGTGT	TCATATGCCA	480
AGTACGCC	CTATTGACGT	CAATGACGGT	AAATGGCCCG	CCTGGCATT	TGCCCAGTAC	540
ATGACCTTAT	GGGACTTTTC	TAATGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	600
ATGGTGATGC	GGTTTTGGCA	GTACATCAAT	GGGGCGTGGAT	AGCGGTTGA	CTCACGGGGA	660
TTTCCAAGTC	TCCACCCCAT	TGACGTCAT	GGGAGTTGT	TTTGGCACCA	AAATCAACGG	720
GACTTCCAA	AATGTCGTA	CAACTCCGCC	CCATTGACGC	AAATGGCCG	TAGGCGTGT	780
CGGTGGGAGG	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTAACTG	840
GCTTATCGAA	ATGTCGACTG	AGAACTTCAG	GGTGAGTTG	GGGACCCCTG	ATTGTTCTTT	900
CTTTTCGCT	ATGTAAAAT	TCATGTTATA	TGGAGGGGC	AAAGTTTCA	GGGTGTTGTT	960
TAGAATGGGA	AGATGTCCTC	TGTATCACCA	TGGACCCCTA	TGATAATT	TTTCTTTCA	1020
CTTTCTACTC	TGTTGACAA	CATTGTCCTC	TCTTATTTC	TTTCATTTT	CTGTAACTT	1080
TTCGTTAAC	TTTAGCTTGC	ATTGTAAACG	AATTTTAAA	TTTCACTTTG	TTTATTGTC	1140
AGATTGTAAG	TACTTTCTCT	AATCACTTTT	TTTCAAGGC	AATCAGGGT	TATTAATTG	1200
TACTTCAGCA	CAGTTTTAGA	GAACAATTGT	TATAATTAA	TGATAAGGTA	GAATATTCT	1260
GCATATAAAAT	TCTGGCTGGC	GTGGAATAT	TCTTATTGGT	AGAAACAAC	ACATCCTGGT	1320
CATCATCTG	CTTTCTCTT	TATGGTACA	ATGATATACA	CTGTTTGAGA	TGAGGATAAA	1380
ATACTCTGAG	TCCAAACCGG	GCCCTCTGC	TAACCATGTT	CATGCCTCT	TCTTTTCCCT	1440
ACAGCTCTG	GGCAACGTGC	TGGTTGTTG	GCTGCTCAT	CATTGCGCA	AGAATTGGCC	1500
GCAAGCTCT	GCAGCATCGT	TCTGTTGTTG	CTCTGCTGA	CTGTGTTCT	GTATTGTC	1560
GAGAATATGG	GCCAGACTGT	TACCACTCCC	TTAAGTTGA	CCTTGGTCA	CTGGGAAAGAT	1620
GTCGAGCGGA	TCGCTCACAA	CCAGTCGGTA	GATGTCAAGA	AGAGACGTG	GGTTACCTTC	1680
TGCTCTGCAG	AATGGCCAAC	CTTTAACGTC	GGATGGCCGC	GAGACGGCAC	CTTTAACCGA	1740
GACCTCATCA	CCCAAGGTTAA	GATCAAGGTC	TTTCACCTG	GCCCCGATGG	ACACCCAGAC	1800
CAGGTCCTCT	ACATCGTGC	CTGGGAACCC	TTGGCTTTG	ACCCCCCTC	CTGGGTCAG	1860
CCCTTGTAC	ACCTTAAGCC	TCCGCCCTC	CTTCCCTCCAT	CGGCCCCGTC	TCTCCCCCTT	1920
GAACCTCCCTC	GTTCGACCCCC	GCCTCGATCC	TCCCTTATC	CAGCCCTCAC	TCCCTCTCTA	1980
GGCGCCAAAC	CTAAACCTCA	AGTTCTTCT	GACAGTGGGG	GGCCGCTCAT	CGACCTACTT	2040
ACAGAAAGACC	CCCCGCCTTA	TAGGGACCCA	AGACCACCC	CTTCCGACAG	GGACGGAAT	2100
GGTGGAGAAG	CGACCCCTGC	GGGAGAGGCA	CCGGACCCCT	CCCCAATGGC	ATCTGCCCTA	2160
CGTGGGAGAC	GGGAGCCCCC	TGTGGCCGAC	TCCACTACCT	CGCAGGCA	CCCCCTCCGC	2220
GCAGGGAGAA	ACGGACAGCT	TCAATACGG	CCGTTCTCT	CTTCTGACCT	TTACAACCTG	2280
AAAAATAATA	ACCCCTCTT	TTCTGAAGAT	CCAGGTAAC	TGACGCTCT	GATCGAGTCT	2340
GTTCTCATCA	CCCATCAGCC	CACCTGGGAC	GACTGTCAGC	AGCTGTTGGG	GACTCTGCTG	2400
ACCGGAGAAG	AAAAACAACG	GGTGCTCTTA	GAGGCTAGAA	AGGCGGTGCG	GGGCGATGAT	2460
GGGCGCCCA	CTCAACTGCC	CAATGAAGTC	GATGCCGTT	TTCCCTCGA	GCGCCAGAC	2520
TGGGATTACA	CCACCCAGGC	AGGTAGGAAC	CACCTAGTC	ACTATGCCA	GTTGCTCCTA	2580
GCGGGTCTCC	AAAACGCGGG	CAGAACCCCC	ACCAATTGG	CCAAGGTA	AGGAATAACA	2640
CAAGGGCCA	ATGAGTCTCC	CTCGGCCCTC	CTAGAGAAC	TTAAGGAAGC	CTATCGCAGG	2700
TACACTCTT	ATGACCCCTGA	GGACCCAGGG	CAAGAACACT	ATGTTGCTAT	GTCTTTCTT	2760
TGGCAGTCTG	CCCCAGACAT	TGGGAGAAAG	TTAGAGAGGT	TAGAAGATT	AAAAAACAAAG	2820
ACGCTTGGAG	ATTTGGTTAG	AGAGGCAGAA	AAGATCTTTA	ATAAACGAGA	AAACCCGGAA	2880
GAAAGAGAGG	AACGTATCAG	GAGAGAAACA	GAGGAAAAAG	AAGAACGCCG	TAGGACAGAG	2940
GATGAGCAGA	AAGAGAAAAGA	AAGAGATCGT	AGGAGACATA	GAGAGATGAG	CAAGCTATTG	3000
GCCACTGTG	TTAGTGGACA	GGAAACAGGAT	AGACAGGGAG	GAGAACGAAG	GAGGTCCTAA	3060
CTCGATCGCG	ACCACTGTC	CTACTGCAA	GAAAAGGGG	ACTGGGCTAA	AGATTGTCCTC	3120
AAGAAACCAC	GAGGCCCTCG	GGGACCAAGA	CCCCAGACCT	CCCTCCTGAC	CCTAGATGAC	3180
TAGGGAGGTC	AGGGTCAGGA	GGCCCCCCT	GAACCCAGGA	TAACCCCTCAA	AGTCGGGGGG	3240
CAACCGTCA	CCTTCCTGGT	AGATACTGGG	GCCCCAACACT	CCGTGCTGAC	CCAAATCCT	3300
GGACCCCTAA	GTGATAAGTC	TGCCCTGGTC	CAAGGGCTA	CTGGAGGAAA	GCGGTATCGC	3360
TGGACCAACGG	ATCGCAAGT	ACATCTAGCT	ACCGGTAAGG	TCACCCACTC	TTTCCTCCAT	3420
GTACCAAGCT	GTCCCTATCC	TCTGTTAGGA	AGAGATTG	TGACTAAAC	AAAAGCCCAA	3480
ATCCACTTTG	AGGGATCAGG	AGCTCAGGTT	ATGGGACCAA	TGGGGCAGCC	CCTGCAAGTG	3540
TTGACCTAA	ATATAGAAGA	TGAGCATCGG	CTACATGAGA	CCTCAAAAGA	GCCAGATGTT	3600
TCTCTAGGGT	CCACATGGCT	GTCTGATTT	CCTCAGGCCT	GGGCGGAAC	CGGGGGCATG	3660
GGACTGGCAG	TTCGCCAAGC	TCCTCTGATC	ATACCTCTGA	AAGCAACCTC	TACCCCGTG	3720
TCCATAAAAC	AATACCCCAT	GTCACAAGAA	GCCAGACTGG	GGATCAAGCC	CCACATACAG	3780
AGACTGTTGG	ACCAAGGGAA	ACTGGTACCC	TGCCAGTCCC	CCTGGAACAC	GCCCCCTGCTA	3840
CCCGTTAAGA	AACCAGGGAC	TAATGATT	AGGCTGTCC	AGGATCTGAG	AGAAGTCAAC	3900
AAGCGGGTGG	AAGACATCCA	CCCCACCGTG	CCCAACCCCTT	ACAACCTCTT	GAGCGGGCTC	3960
CCACCGTCCC	ACCACTGGTA	CACTGTGCTT	GATTAAAGG	ATGCTTTTT	CTGCTTGAGA	4020
CTCCACCCCA	CCACTCAGCC	TCTCTCGCC	TTTGAATGGA	GAGATCCAGA	GATGGGAATC	4080

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Figure 7. hCMV+intron Sequence

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TCAGGACAAT	TGACCTGGAC	CAGACTCCC	CAGGGTTTCA	AAAACAGTCC	CACCCTGTTT	4140
GATGAGGCAC	TGCACAGAGA	CCTAGCAGAC	TTCCGGATCC	AGCACCCAGA	CTTGATCCTG	4200
CTACAGTACG	TGGATGACTT	ACTGCTGGCC	GCCACTTCTG	ACCTAGACTG	CCAACAAGGT	4260
ACTCGGGCCC	TGTTACAAAC	CCTAGGAAC	CTCGGGTATC	GGGCGCTGGC	CAAGAAAGCC	4320
CAAATTGCGC	AGAAACAGGT	CAAGTATCTG	GGGTATCTTC	TAAGAGAGGG	TCAGAGATGG	4380
CTGACTGAGG	CCAGAAAAGA	GACTGTGATG	GGGCAGCCTA	CTCCGAAGAC	CCCTCGACAA	4440
CTAAGGGAGT	TCCTAGGGAC	GGCAGGCTTC	TGTCGCTCT	GGATCCCTGG	GTTTCAGAA	4500
ATGGCAGCCC	CCTTGTACCC	TCTCACAAA	ACGGGGACTC	TGTTTAATTG	GGGCCAGAC	4560
CAACAAAAGG	CCTATCAAGA	AATCAAGCAA	GCTCTTCTAA	CTGCCCCAGC	CCTGGGGTTG	4620
CCAGATTGTA	CTAAGCCCTT	TGAACCTTTT	GTGACGAGA	AGCAGGGCTA	CGCCAAAGGT	4680
GTCCTAACGC	AAAAAACTGGG	ACCTTGCGT	CGGCCGGTGG	CCTACCTGTC	AAAAAAGCTA	4740
GACCCAGTAG	CAGCTGGGTG	GCCCCCTTGC	CTACGGATGG	TAGCAGCCAT	TGCGCTACTG	4800
ACAAAGGATG	CAGGCAAGCT	AACCATGGGA	CAGGGCACTC	TCATTCTGGC	CCCCCATGCA	4860
GTAGAGGCAC	TAGTCAAACAA	ACCCCCCGAC	CGCTGGCTTT	CCAAACGCCC	GATGACTCAC	4920
TATCAGGCCT	TGCTTTTGGG	CACGGACCGG	GTCCAGTTCG	GACCGGTGGT	AGCCTCTAAC	4980
CCGGCTACGC	TGCTCCCACT	GCCTGAGGAA	GGGCTGCAAC	ACAAC TGCT	TGATATCCTG	5040
GCCGAAGCCC	ACGGAACCCCG	ACCCGACCTA	ACGGACCAGC	CGCTCCCAGA	CGCCGACCAC	5100
ACCTGGTACA	CGGATGGAA	CAGTCTCTTA	CAAGAGGGAC	AGCGTAAGGC	GGGAGCTGCG	5160
GTGACCACCG	AGACCGAGGT	AATCTGGCT	AAAGCCCTGC	CAGCGGGGAC	ATCCCCTCAG	5220
CGGGCTGAAC	TGATAGCACT	CACCCAGGCC	CTAAAGATGG	CAGAAGGTTAA	GAAGCTAAAT	5280
GTTTATACTG	ATAGCCGTTA	TGCTTTTGCT	ACTGCCCATA	TCCATGGAGA	AATATACAGA	5340
AGGCGTGGGT	TGCTCACATC	AGAAGGAAA	GAGATCAAA	ATAAAAGACGA	GATCTTGGCC	5400
CTACTAAAAG	CCCTCTTCT	GCCAAAAGA	CTTAGCATAA	TCCATTGTC	AGGACATCAA	5460
AAGGGACACA	GGCGCGAGGC	TAGAGGCAAC	CGGATGGCTG	ACCAAGCGGC	CCGAAAGGCA	5520
GCCATCACAG	AGACTCCAGA	CACCTCTACC	CTCCCTCATAG	AAAATTCTAC	ACCCCTACACC	5580
TCAGAACATT	TTCATTACAC	AGTGAATGAT	ATAAAGGACC	TAACCAAGTT	GGGGGCCATT	5640
TATGATAAAA	CAAAGAAGTA	TTGGCTCTAC	CAAGGGAAAAC	CTGTGATGCC	TGACCAAGTTT	5700
ACTTTGAAAT	TATTAGACTT	TCTTCATCAG	CTGAACCTACC	TCAGCTTCTC	AAAAATGAAG	5760
GCTCTCTAG	AGAGAAGCCA	CAGTCCCTAC	TACATGCTGA	ACCGGGATCG	AAACACTCAA	5820
AATATCACTG	AGACCTGCAA	AGCTTGTGCA	CAAGTCAAACG	CCAGCAAGTC	TGCCGTTAAA	5880
CAGGGAACAA	GGGTCCCGGG	GCATCGGCC	GGCACTCATT	GGGAGATCGA	TTTCACCGAG	5940
ATAAAAGCCCC	GATTGTATGG	CTATAAAATAT	CTTCTAGTTT	TTATAGATAC	CTTTCTGGC	6000
TGGATAGAAG	CCTTCCCAAC	CAAGAAAGAA	ACCCCAAGG	TCGTAACCAA	GAAGCTACTA	6060
GAGGAGATCT	TCCCCAGGTT	CGGCATGCC	CAGGTATTGG	GAACGTACAA	TGGGCTGCGC	6120
TTCGTCCTCA	AGGTGAGTCA	GACAGTGGCC	GATCTGTTGG	GGATTGATTG	GAAATTACAT	6180
TGTGCATACA	GACCCCAAAG	CTCAGGCCAG	GTAGAAAGAA	TGAATAGAAC	CATCAAGGAG	6240
ACTTTAACTA	AATTAACGCT	TGCAACTGGC	TCTAGAGACT	GGGTGCTCCT	ACTCCCCTTA	6300
GCCCTGTAC	GGGCCCGCAA	CACGCCGGGC	CCCCATGGCC	TCACCCCAT	TGAGATCTTA	6360
TATGGGGCAC	CCCCGCCCCCT	TGTAAACTTC	CCTGACCCCTG	ACATGACAAG	AGTTACTAAC	6420
AGCCCCCTCTC	TCCAAGCTCA	CTTACAGGCT	CTCTACTTAG	TCCAGCACGA	AGTCTGGAGA	6480
CCTCTGGCGG	CAGCCTACCA	AGAACACTG	GACCGACCGG	TGGTACCTCA	CCCTTACCGA	6540
GTCGGCGACA	CAGTGTGGGT	CCGCGCACAC	CAGACTAAGA	ACCTAGAAC	TCGCTGGAAA	6600
GGACCTTACA	CAGTCCTGCT	GACCACCCCC	ACCGCCCTCA	AAGTAGACGG	CATCGCAGCT	6660
TGGATACACG	CCGCCCCACGT	GAAGGCTGCC	GACCCCGGGG	GTGGACCATC	CTCTAGACTG	6720
ACATGGCGCG	TTCAACGCTC	TCAAAACCCC	TTAAAATAA	GGTTAACCGG	CGAGGGCCCC	6780
TAATCCCCCT	AATTCTCTG	ATGCTCAGAG	GGGTCACTAC	TGCTTCGCCC	GGCTCCAGTG	6840
CGGCCCAAGCC	GGCCACCATG	AAAACATTA	ACATTCTCA	ACAAGATCTA	GAATTAGTAG	6900
AAGTAGCGAC	AGAGAAGATT	ACAATGCTT	ATGAGGATAA	TAAACATCAT	GTGGGAGCGG	6960
CAATTCTGAC	GAAAACAGGA	GAAATCATTT	CGGCAGTACA	TATTGAAGCG	TATATAGGAC	7020
GAGTAACGTG	TTGTGCAGAA	GCCATTGCCA	TTGGTAGTGC	AGTTTCGAAT	GGACAAAAGG	7080
ATTTGACAC	GATTGTAGCT	GTTAGACACC	CTTATTCTGA	CGAAGTAGAT	AGAAGTATTG	7140
GAGTGGTAAG	TCCCTGTGGT	ATGTGTAGGG	AGTTGATTTC	AGACTATGCA	CCAGATTGTT	7200
TTGTGTTAAT	AGAAAATGAAT	GGCAAGTTAG	TCAAAACTAC	GATTGAAGAA	CTCATTCAC	7260
TCAAATATAC	CGGAAATTAA	AAGTTTAC	ACCAAGCTTA	TCGAATTTC		7308

Figure 8. hCMV+intronkaSD Sequence

1

AGATCTCCCG	ATCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	60
AGCCAGTATC	TGCTCCCTGC	TTGTGTGTTG	GAGGTGCGTG	AGTAGTGCAC	GAGCAAAATT	120
TAAGCTACAA	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	180
CGTTTGCAGC	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	240
AGTTATTAAT	AGTAATCAAT	TACGGGGTCA	TTAGTTCAT	GCCCCATATAT	GGAGTTCCGC	300
GTTACATAAC	TTACCGTAAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCATTG	360
ACGTCAATAA	TGACGTATGT	TCCCAGTAGA	ACCCCAATAG	GGACTTTCCA	TTGACGTCAA	420
TGGGGTGGACT	ATTTACGCTTA	AACTGCCCCAC	TTGGCAGTAC	ATCAAGTGT	TCATATGCCA	480
AGTACGCCCC	CTATTGACGT	CAATGACGGT	AAATGGCCCG	CCTGGCATTA	TGCCCAGTAC	540
ATGACCTTAT	GGGACTTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	600
ATGGTGTGTC	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	ACCGGTTG	CTCACGGGG	660
TTTCCAAGTC	TCCACCCCAT	TGACGTCAT	GGGAGTTGT	TTTGGCACCA	AAATCAACGG	720
GACTTCCAA	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGCCG	TAGGCGTGT	780
CGGGGGGAGG	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTAAC	840
GCTTATCGAA	ATGTCGACTG	AGAACCTCAG	GGTAGGTTTG	GGGACCCCTG	ATTGTTCTTT	900
CTTTTCGCT	ATTGAAAAAT	TCATGTTATA	TGGAGGGGGC	AAAGTTTCA	GGGTGTTGTT	960
TAGAATGGGA	AGATGTCCTC	TGTATCACCA	TGGACCTCTA	TGATAATT	TTTCTTTCA	1020
CTTTCTACTC	TGTTGACAAC	CATTGCTCTC	TCTTATTTTC	TTTTCATT	CTGTAAC	1080
TTCGTTAAC	TTAGCTTGC	ATTGTAACG	AATTTTAAA	TTCACTTTG	TTTATTTGTC	1140
AGATTGTAAG	TACTTCTCT	AATCACTTT	TTTCAAGGC	AATCAGGTA	TATTATATTG	1200
TACTTCAGCA	CAGTTTTAGA	GAACAATTGT	TATAATTAAA	TGATAAGGTA	GAATATTCT	1260
GCATATAAAAT	TCTGGCTGGC	GTGGAATAT	TCTTATTGGT	AGAAAACA	ACATCCTGGT	1320
CATCATCCTG	CCTTCTCTT	TATGGTACA	ATGATATACA	CTGTTGAGA	TGAGGATAAA	1380
ATACTCTGAG	TCCAAACCGG	GCCCCCTGTC	TAACCATGTT	CATGCCCT	TCTTTTCCT	1440
ACAGCTCC	GGCAACGTGC	TGGTTGTTGT	GCTGTCAT	CATTGGCCA	AGAATTGGCC	1500
GCAAGCTCT	GCAGCATCGT	TCTGTGTTGT	CTCTGTCAT	CTGTGTTCT	GTATTGTC	1560
GAGAATATGG	GCCAGACTGT	TACCACTCCC	TTAAGTTGA	CCTTGGTCA	CTGGAAAGAT	1620
GTCGAGCGGA	TCGCTCACAA	CCAGTCGGTA	GATGTCAGA	AGAGACGTG	GGTTACCTTC	1680
TGCTCTGAG	AATGGCCAAAC	CTTTAACGTC	GGATGGCGC	GAGACGGC	CTTTAACCGA	1740
GACCTCATCA	CCCCAGGTTAA	GATCAAGGT	TTTCACCTG	GCCCCGATGG	ACACCCAGAC	1800
CAGGTTCCCT	ACATCGTGC	CTGGGAAGCC	TTGGCTTTG	ACCCCCCTC	CTGGGTCAAG	1860
CCCTTGTAC	ACCCCTAACCC	TCCGCCCTC	CTTCCCTCCAT	CCGCCCTGTC	TCTCCCCCTT	1920
GAACCTCCTC	GTCGACCCCC	GCCTCGATCC	TCCCTTATC	CAGCCCTCAC	TCCTTCTCTA	1980
GGCGCCAAAC	CTAAACCTCA	AGTTCTTCT	GACAGTGGGG	GGCCGCTCAT	CGACCTACTT	2040
ACAGAAGACC	CCCCGCCTTA	TAGGGACCCA	AGACCACCCC	CTTCCGACAG	GGACGGAAAT	2100
GGTGGAGAAG	CGACCCCTGC	GGGAGGGCA	CCGGACCCCT	CCCCAATGGC	ATCTCGCTA	2160
CGTGGAGAGC	GGGAGCCCCA	TGTGGCCGAC	TCCACTACCT	CCGAGGCA	CCCCCTCCGC	2220
GCAGGAGGAA	ACGGACAGCT	TCAAACTCTG	CCGTTCTCT	CTTCTGACT	TTACAAC	2280
AAAAATAATA	ACCCCTCTTT	TTCTGAAGAT	CCAGGTAAC	TGACAGCT	GATGGAGTCT	2340
GTTCTCATCA	CCCACATCAGCC	CACCTGGGAC	GACTGTCAGC	AGCTGTTGGG	GACTCTGCTG	2400
ACCGGAGAAG	AAAAACAAACG	GGTGCTCTTA	GAGGCTAGAA	AGGCCTGCG	GGGCGATGAT	2460
GGGCGCCCA	CTCAACTGCC	CAATGAAGTC	GATGCCGCTT	TTCCCTCGA	GCGCCAGAC	2520
TGGGATTACA	CCACCCAGGC	AGGACGCAAC	CACCTAGTC	ACTATCGCA	GTGCTCC	2580
GCGGGTCTCC	AAAACGCGGG	CAGAAGCCCC	ACCAATTGG	CCAAAGGTA	AGGAATAACA	2640
CAAGGGCCCA	ATGAGTCTCC	CTCGGCCCTC	CTAGAGAGAC	TTAAGGAAGC	CTATCGCAGG	2700
TACACTCCTT	ATGACCCCTGA	GGACCCAGGG	CAAGAAACTA	ATGTGTCTAT	GTCTTTCATT	2760
TGGCAGTCTG	CCCCAGACAT	TGGGAGAAAG	TTAGAGAGGT	TAGAAGATT	AAAAAAACAAG	2820
ACGCTTGGAG	ATTGGTTAG	AGAGGCAGAA	AAGATCTTA	ATAAAACGAGA	AAACCCGGAA	2880
GAAAGAGAGG	AACTGATCTAG	GAGAGAAACA	GAGGAAAAG	AAGAACGCG	TAGGACAGAG	2940
GATGAGCAGA	AAAGAGAAAAGA	AAAGAGATCGT	AGGAGACATA	GAGAGATGAG	CAAGCTATTG	3000
GCCACTGTCG	TTAGTGGAC	GAACAGGAT	AGACAGGGAG	GAGAACGAGA	GAGGCCCCAA	3060
CTCGATCGCG	ACCACTGTC	CTACTGCAA	AAAAAGGGGC	ACTGGGCTAA	AGATTGTC	3120
AAGAAACCAC	GAGGACCTCG	GGGACCAAGA	CCCCAGACCT	CCCTCCTGAC	CCTAGATGAC	3180
TAGGGAGGTC	AGGGTCAGGA	GCCCCCCCC	GAACCCAGGA	TAACCCCTCAA	AGTCGGGGGG	3240
CAACCCGTC	CCTTCTCTGGT	AGATACTGGG	GCCCCAACACT	CCGTGCTGAC	CCAAATACCT	3300
GGACCCCTAA	GTGATAAGTC	TGCCCTGGTC	CAAGGGCTA	CTGGAGGAAA	GGGGTATCGC	3360
TGGACCAAGG	ATCGCAAAGT	ACATCTAGCT	ACGGGTAAAG	TCACCCACTC	TTTCTCCAT	3420
GTACCAAGACT	GTCCCTATCC	TCTGTTAGGA	AGAGATTGTC	TGACTAAAC	AAAAGCCAA	3480
ATCCACTTTG	AGGGATCAGG	AGCTCAGGTT	ATGGGACAA	TGGGGCAGG	CCTGCAAGTG	3540
TTGACCCCTAA	ATATAGAAGA	TGACCATCGG	CTACATGAGA	CCTCAAAAGA	GCCAGATGTT	3600
TCTCTAGGGT	CCACATGGCT	GTCTGATT	CCTCAGGCCT	GGGCGGAAAC	CGGGGGCATG	3660
GGACTGGCAG	TTCGCAAGC	TCCTCTGATC	ATACCTCTGA	AAGCAACCTC	TACCCCGTG	3720
TCCATAAAAC	AAATCCCCAT	GTCACAAAGAA	GCCAGACTGG	GGATCAAGCC	CCACATACAG	3780
AGACTGTTGG	ACCAAGGAAT	ACTGGTACCC	TGCCAGTCCC	CCTGGAACAC	GCCCCCTGCTA	3840
CCCGTTAAGA	AACCAGGGAC	TAATGATTAT	AGGGCTGTCC	AGGATCTGAG	AGAACGCAAC	3900
AAGCGGGTGG	AAGACATCCA	CCCCACCGTG	CCCAACCCCT	ACAACCTCTT	GAGCAGGGCTC	3960
CCACCGTCCC	ACCAGTGGTA	CACTGTGCTT	GATTTAAAGG	ATGCCTTTT	CTGCGCTGAGA	4020
CTCCACCCCA	CCAGTCAGCC	TCTCTCGCC	TTTGAGTGG	GAGATCCAGA	GATGGGAATC	4080

Figure 8. hCMV+intronkaSD Sequence

TCAGGACAAT	TGACCTGGAC	CAGACTCCC	CAGGGTTTCA	AAAACAGTCC	CACCCCTGTT	4140
GATGAGGCAC	TGCACAGAGA	CCTAGCAGAC	TTCCGGATCC	AGCACCCAGA	CTTGATCCTG	4200
CTACAGTACG	TGGATGACTT	ACTGCTGGCC	GCCACTTCTG	AGCTAGACTG	CCAACAAGGT	4260
ACTCGGGCCC	TGTTACAAAC	CCTAGGGAAAC	CTCGGGTATC	GGGCCTCGGC	CAAGAAAGCC	4320
CAAATTGAGG	AGAAACAGGT	CAAGTATCTG	GGGTATCTC	AAAAGAGGG	TCAGAGATGG	4380
CTGACTGAGG	CCAGAAAAGA	GACTGTGATG	GGGCAGGCTA	CTCCGAAGAC	CCCTCGACAA	4440
CTAAGGGAGT	TCCTAGGGAC	GGCAGGCTTC	TGTCGCCTC	GGATCCCTGG	GTTTCAGAA	4500
ATGGCAGCCC	CCTTGTACCC	TCTCACAAA	ACGGGGACTC	TGTTTAATTG	GGGCCAGAC	4560
CAACAAAAGG	CCTATCAAGA	AATCAAGCA	CCTCTTCTAA	CTGCCCCAGC	CCTGGGGTTG	4620
CCAGATTGAG	CTAAGCCCTT	TGAACCTTT	GTCGACGAGA	AGCAGGGCTA	CGCCAAAGGT	4680
GTCCTAACGC	AAAAACTGGG	ACCTTGGCGT	CGGCCGGTGG	CCTACCTGTC	AAAAAAGCTA	4740
GACCCAGTAG	CAGCTGGGTG	GCCCCCTTGC	CTACGGATGG	TAGCAGCCAT	TGCCGTACTG	4800
ACAAAGGATG	CAGGCAAGCT	AACCATGGGA	CAGCCACTAG	TCATTCTGGC	CCCCCATGCA	4860
GTAGAGGCAC	TAGTCAAACAA	ACCCCCCGAC	CGCTGGCTTT	CCAACCGCCG	GATGACTCAC	4920
TATCAGGCC	TGCTTTTGGA	CACGGACCGG	GTCCAGTTCG	GACCGGTGGT	AGCCCTGAAC	4980
CCGGCTACGC	TGCTCCCAC	GCCTGAGGAA	GGGCTGCAAC	ACAAC TGCT	TGATATCCTG	5040
GCCGAAGCCC	ACCGAACCCG	ACCCGACCTA	ACGGACCAGC	CGCTCCAGA	CGCCGACCAC	5100
ACCTGGTACA	CGGATGGAAG	CAGTCTCTTA	CAAGAGGGAC	AGCGTAAGGC	GGGAGCTGCG	5160
GTGACCAACG	AGACCGAGGT	AATCTGGCT	AAAGCCCTGC	CAGCCGGAC	ATCCGCTCAG	5220
CGGGCTGAAC	TGATAGCACT	CACCCAGGCC	CTAAAGATGG	CAGAAGGTAA	GAAGCTAAAT	5280
GTTTATACTG	ATAGCCGTTA	TGCTTTTGTG	ACTGCCCTA	TCCATGAGA	AATATACAGA	5340
AGGCGTGGGT	TGCTCACATC	AGAAGGAAA	GAGATCAAAA	ATAAAAGACGA	GATCTTGGCC	5400
CTACTAAAAG	CCCTCTTTCT	GCCCCAAAGA	CTTAGCATAA	TCCATTGTCC	AGGACATCAA	5460
AAGGGACACAA	GCGCCGAGGC	TAGAGGCAAC	CGGATGGCTG	ACCAAGGGC	CCGAAAGGCA	5520
GCCATCACAG	AGACTCCAGA	CACCTCTACC	CTCCTCATAG	AAAATTCA	ACCCATACACC	5580
TCAGAACATT	TTCAATTACAC	AGTGACTGAT	ATAAAGGACC	TAACCAAGTT	GGGGCCATT	5640
TATGATAAAA	CAAAGAAGTA	TTGGGCTAC	CAAGGAAAAC	CTGTGATGCC	TGACCAGTTT	5700
ACTTTGAAAT	TATTAGACTT	TCTTCATCAG	CTGACTCACC	TCAGCTTCTC	AAAAATGAAG	5760
GCTCTCTTAG	AGAGAAGCCA	CAGTCCCAC	TACATGCTGA	ACCGGGATCG	AACACTCAA	5820
AATATCACTG	AGACCTGCAA	AGCTTGTC	CAAGTCAACG	CCAGCAAGTC	TGCCGTTAAA	5880
CAGGGAACTA	GGGTCCCGCG	GCATGGCCC	GGCACTCATT	GGGAGATCGA	TTTCACCGAG	5940
ATAAAAGCCG	GATTGTATGG	CTATAAATAT	CTTCTAGTTT	TTATAGATAC	CTTTCTGGC	6000
TGGATAGAAG	CCTTCCCAAC	CAAGAAAGAA	ACGCCAAGG	TCGTAACCAA	GAAGCTACTA	6060
GAGGAGATCT	TCCCCCAGGT	CGGCATGCC	CAGGTATTGG	GAACCTGACAA	TGGGCTGCC	6120
TTCGTCTCCA	AGGTGAGTC	GACAGTGGCC	GATCTGTTGG	GGATTGATTG	GAATATTACAT	6180
TGTGCATACA	GACCCCAAAG	CTCAGGGCAG	GTAGAAAGAA	TGAATAGAAC	CATCAAGGAG	6240
ACTTTAACTA	AAATTACGCT	TGCAACTGGC	TCTAGAGACT	GGGTGCTCT	ACTCCCCTTA	6300
GCCCTGTACC	GAGCCCCCAA	CACGCCGGC	CCCCATGGCC	TCACCCCCATA	TGAGATCTTA	6360
TATGGGGCAC	CCCCGCCCCCT	TGTAAACCTC	CCTGACCCCTG	ACATGACAAG	AGTTACTAAC	6420
AGCCCCCTCTC	TCCAAGCTCA	CTTACAGGCT	CTCTACTTAG	TCCAGCACGA	AGTCTGGAGA	6480
CCTCTGGCGG	CAGCCTACCA	AGAACAACTG	GACCGACCAGG	GGGTACCTCA	CCCTTACCGA	6540
GTCGGCGACA	CAGTGTGGGT	CGGCCGACAC	CAGACTAAGA	ACCTAGAAC	TGCGCTGGAAA	6600
GGACCTTACA	CAGTCTCTG	GACCAACCCC	ACGGCCCTCA	AAGTAGACGG	CATCCGAGCT	6660
TGGATACACG	CGGCCAACGT	GAAGGCTGCC	GACCCCCGGGG	GTGGACCATC	CTCTAGACTG	6720
ACATGGCGCG	TTCAACGCTC	TCAAAACCCC	TTAAAAATAA	GGTTAACCCG	CGAGGCCCCC	6780
TAATCCCCTT	AAATTCTCTG	ATGCTCAGAG	GGGTCACTAC	TGCTTCGCCC	GGCTCCAGTG	6840
CGGCCAGCC	GGCCACCATG	AAAACATTAA	ACATTCTCA	ACAAGATCTA	GAATTAGTAG	6900
AAGTAGCGAC	AGAGAACAGT	ACAATGCTT	ATGAGGATAA	TAAACATCAT	GTGGGAGCGG	6960
CAATTCTGTAC	GAAAACAGGA	GAAATCATTT	CGGCAGTACA	TATTGAAGCG	TATATAGGAC	7020
GAGTAACGTG	TTGTGCAAGA	GCCATTGCGA	TTGGTAGTGC	AGTTTCGAAT	GGACAAAAGG	7080
ATTTTGACAC	GATTGTAGCT	GTTAGACACC	CTTATTCTGA	CGAAGTAGAT	AGAAGTATTTC	7140
GAGTGGTAAG	TCCTTGTGGT	ATGTGTAGGG	AGTTGATTTC	AGACTATGCA	CCAGATTGTT	7200
TTGTGTTAAT	AGAAATGAAT	GGCAAGTTAG	TCAAAACTAC	GATTGAAGAA	CTCATTCCAC	7260
TCAAATATAC	CCGAAATTAA	AAGTTTAC	ACCAAGCTTA	TCGAATTTC		7308

Figure 9. FBdelPASAF Sequence

CATATGCGGT	GTGAAATACC	GCACAGATGC	CTAAGGAGAA	AATACCGCAT	CAGGGGCCAT	60
TCGCCATTCA	GGCTGCGCAA	CTGTTGGGAA	GGGCGATCGG	TGGGGGCCCT	TTCGCTATTA	120
CGCCAGCTGG	CGAAAGGGGG	ATGTGCTGCA	AGGCAGATTAA	GTTGGGTAAC	GCCAGGGTTT	180
TCCCAGTCAC	GACGTTGTAA	AACGACGGCC	AGTGAATTCC	GATTAGTTCA	ATTGTTAAA	240
GACAGGATCT	CAGTAGTCGA	GGCTTTAGTC	CTGACTCAAC	AATACCAACCA	GCTAAAACCA	300
CTAGAATACG	AGCCACAATA	AATAAAAGAT	TTTATTAGT	TTCCAGAAAA	AGGGGGGAAT	360
GAAAGACCCC	ACCAAATTCG	TTAGCCTGAT	AGCCGAGTA	ACGCATTTT	GCAAGGCATG	420
GAAAATACCC	AAACCAAGAA	TAGAGAAGTT	CAGATCAAGG	GCGGGTACAC	GAAAACAGCT	480
AACGTTGGC	CAAACAGGAT	ATCTGCGGTG	AGCAGTTTCG	GCGGGGGCCC	GGGGCCAAGA	540
ACAGATGGTC	ACCGCGGTT	GGCCCCGGCC	CGGGGCCAAG	AACAGATGGT	CCCCAGATAT	600
GGCCCAACCC	TCAGCAGTTT	CTTAAGACCC	ATCAGATGTT	TCCAGGCTCC	CCCAAGGACC	660
TGAAATGACC	CTGTGCTTAA	TTTGAATTAA	CCAATCAGCC	TGCTTCTCGC	TTCTGTTCGC	720
GCGCTCTGC	TTCCCGAGCT	CTATAAAAGA	GCTACAACCC	CCTCACTCGG	CGCGCCAGTC	780
CTCCGATAGA	CTGAGTCGCC	CGGGTACCCG	TGTATCCAAT	AAATCCTCTT	GCTGTTGCAT	840
CCGACTCGT	GTCCTCGTGT	TCCTTGGGAG	GGTCTCCTCA	GAGTGAATG	CTACCCGTCT	900
CGGGGGTCTT	TCATTTGGGG	GCTCGTCCGG	GATCTGGAGA	CCCCCTGCCA	GGGACCCACCG	960
ACCCACCACC	GGGAGGTAAG	CTGGCCAAGA	TCTTATATGG	GGCACCCCCG	CCCCCTGTAA	1020
ACTTCCCTGA	CCCTGACATG	ACCAGAGTTA	CTAACAGGCC	CTCTCTCCAA	GCTCACTTAC	1080
AGGCTCTCTA	CTTAGTCCAG	CACGAAGTTT	GGAGACCACT	GGCGGCAGCT	TACCAAGAAC	1140
AACTGGACCG	GCCGGTGGTG	CCTCACCCCT	ACCGGGTCGG	CGACACAGTG	TGGGTCCGCC	1200
GACATCAAAC	AAAGAACCTA	GAACCTCGT	GGAAAGGACC	TTACACAGTC	CTGCTGACCA	1260
CCCCCACCGC	CCTCAAAGTA	GACGGTATCG	CAGCTGGAT	ACACGCAGCC	CACGTAAAGG	1320
CGGCCGACAC	CGGAGATGGA	CCATCCTCG	GACGGACATG	GCGGCTTCAA	CGCTCTCAA	1380
ACCCCTCAA	GATAAGATTA	ACCCGTTGAA	GCCCTTAATA	GTCATGGGAG	TCCTGTTAGG	1440
AGTAGGGATG	GCAGAGAGCC	CCCATCAGGT	CTTTAATGTA	ACCTGGAGAG	TCACCAACCT	1500
GATGACTGGG	CGTACCGCCA	ATGCCACCTC	CCTCCTGGGA	ACTGTACAAG	ATGCCCTCCC	1560
AAAATTATAT	TTTGATCTAT	GTGATCTGGT	CGGAGAGGAG	TGGGACCCCTT	CAGACCAGGA	1620
ACCGTATGTC	GGGTATGGCT	GCAAGTACCC	CGCAGGGAGA	CAGCGGACCC	GGACTTTTG	1680
CTTTTACGTC	TGCCCTGGGG	ATACCGTAA	GTCGGGGTGT	GGGGGACCAAG	GAGAGGGCTA	1740
CTGTGGTAAA	TGGGGGTGTC	AAACCCACGG	ACAGGCTTAC	TGGAAGGCCA	CATCATCGT	1800
GGACCTAATC	TCCCTTAAGC	GCGGTAACAC	CCCCCTGGGAC	ACGGGATGCT	CTAAAGTTGC	1860
CTGTGGCCCC	TGCTACGACC	TCTCCAAAGT	ATCCAATTCC	TTCCAAGGGG	CTACTCGAGG	1920
GGGAGATGC	AACCCCTCTAG	TCCTAGAATT	CACTGATGCA	GGAAAAAAAGG	CTAACTGGGA	1980
CGGGCCAAA	TCGTGGGGAC	TGAGACTGTA	CCGGACAGGA	ACAGATCTA	TTACCATGTT	2040
CTCCCTGACC	CGGCAGGTCC	TTAATGTGGG	ACCCCGAGTC	CCCATAGGGC	CCAACCCAGT	2100
ATTACCCGAC	CAAAGACTCC	CTTCCCTCACC	AATAGAGATT	GTACCGGCTC	CACAGCCACC	2160
TAGCCCCCTC	AATACCAAGT	ACCCCCCTTC	CACTACCACT	ACACCCCTAA	CCTCCCCCTAC	2220
AAAGTCCAAGT	GTCCCCACAGC	CACCCCCCAGG	AACTGGGAGAT	AGACTACTAG	CTCTAGTCAA	2280
AGGAGCCTAT	CAGGCGCTTA	ACCTCACCAA	TCCCGACAAG	ACCCAAGAAAT	GTTGGCTGTG	2340
CTTAGTGTG	GGACCTCCTT	ATTACGAAGG	AGTAGCGGTG	GTGGGCACCTT	ATACCAATCA	2400
TTCCACCGCT	CCGGCCAAC	GTACGGCCAC	TTCCAACAT	AAGCTTACCC	TATCTGAAGT	2460
GACAGGACAG	GGCCTATGCA	TGGGGCAGT	ACCTAAAAC	CACCGAGCCT	TATGTAACAC	2520
CACCCAAAGC	GCCGGCTCAG	GACCTCTACTA	CCTTGCAGCA	CCCGCCGGA	CAATGTGGC	2580
TTGCACT	GGATTGACTC	CCTGCTTGTG	CACCAACGGT	CTCAATCTAA	CCACAGATTA	2640
TTGTGTATTA	GTTGAACCT	GGCCCAGAGT	AATTACAC	TCCCCCGATT	ATATGTATGG	2700
TCAGCTGAA	CAGCGTACCA	AATATAAAAG	AGAGCCAGTA	TCATTGACCC	TGGCCCTTCT	2760
ACTAGGAGGA	TTAACCATGG	GAGGGATTGC	AGCTGGAATA	GGGACGGGGA	CCACTGCCTT	2820
AATTAAAACC	CAGCAGTTTG	AGCAGCTTC	TGCCGCTATC	CAGACAGACC	TCAACGAAGT	2880
CGAAAAGTC	ATTACCAACC	TAGAAAAGTC	ACTGACCTCG	TTGTCTGAAG	TAGTCTACA	2940
GAACCCAGA	GGCTTAGATT	TGCTATTCT	AAAGGAGGGA	GGTCTCTCG	CAGCCCTAAA	3000
AGAAGAAATGT	TGTTTTATG	CAGACACAC	GGGGCTAGTG	AGAGACACCA	TGCCCCAAATT	3060
AAGAGAAAAGG	CTTAATCAGA	GACAAAAC	ATTTGAGACA	GGCCAAGGAT	GGTTCGAAGG	3120
GCTGTTAAT	AGATCCCCCT	GGTTTACAC	CTTAATCTCC	ACCATCATGG	GACCTCTAA	3180
AGTACTCTTA	CTGATCTTAC	TCTTGGACC	TTGCAATTCTC	AATCGATTAG	TTCAATTGTT	3240
TAAAGACAGG	ATCTCAGTAG	TCCAGGCTT	AGTCTCTACT	CAACAATACC	ACCAGCTAAA	3300
GCCTATAGAG	TACGGCCAT	AGGGCGCTA	GTGTTGACAA	TTAATCATCG	GCATAGTATA	3360
CGGCATAGTA	TAATACGACT	CACTATAGGA	GGGGCACCAT	GGCCAAGTTG	ACCAGTGCCTG	3420
TTCCGGTCT	CACCGCGCGC	GACGTGGCCG	GAGGGTGTG	TGTCCTGACC	GACCGGGCTCG	3480
GGTTCTCCCG	GGACTTCGTG	GAGGACGACT	TCGGCGGTGT	GGTCCGGGAC	GACGTGACCC	3540
TGTTCATCAG	CGCGGTCCAG	GACCAGGTGG	TGCCCCACAA	CACCTGGCC	TGGGTGTGGG	3600
TGCGGGCCT	GGACGAGCTG	TACGCCAGT	GGTCGGAGGT	CGTGTCCACG	AACTTCCGGG	3660
ACGCCTCCGG	GCCGGCCATG	ACCGAGATCG	GGGAGCAGCC	GTGGGGCGG	GAGTTCGCCC	3720
TGCGGCCACCC	GGCCGGCAAC	TGCGTGCACT	TCGTGGCCGA	GGAGCAGGAC	TGANNNNCGG	3780
ACGGTCGAC	TTGTTAACCT	GTTTATTGCA	GCTTATAATG	GTTACAAATA	AAGCAATAGC	3840
ATCACAAATT	TCACAAATAA	AGCATTTTT	TCACTGCACT	CTAGTTGTTG	TTTGTCCAAA	3900
CTCATCAATG	TATCTTATCA	TGTCTGGATC	CAGATCTGGG	CCCCATGCGC	CGCGGATCGA	3960
TNNNNACATG	TGAGCAAAG	GCCAGAAAA	GGCCAGGAAC	CGTAAAAAGG	CCGCGTTGCT	4020
GGCGTTTTTC	CATAGGCTCC	GGCCCCCTGA	CGAGCATCAC	AAAAATCGAC	GCTCAAGTCA	4080

Figure 9. FBdelPASAF Sequence

GAGGTGGCGA	AACCCGACAG	GAECTATAAAG	ATACCAGGC	TTTCCCCCTG	GAAGCTCCCT	4140
CGTGCCTCT	CCTGTTCCGA	CCCTGCCGCT	TACCGGATAC	CTGTCCGCC	TTCTCCCTTC	4200
GGGAAGCGTG	GGCCTTTCTC	AATGCTCACG	CTGTAGGTAT	CTCAGTTCGG	TGTAGGTCGT	4260
TCGCTCCAAG	CTGGGCTGTG	TGCACGAACC	CCCGGTTCA	CCCGACCGCT	GCGCCTTATC	4320
CGGTAACAT	CCTCTTGAGT	CCAACCCGGT	AAGACACGAC	TTATCGCAC	TGGCAGCAGC	4380
CACTGGTAAC	AGGATTAGCA	GAGCGAGGT	TGTAGGCCT	GCTACAGAGT	TCTTGAAGTG	4440
GTGGCTAAC	TACGGCTACA	CTAGAAGGAC	AGTATTTGGT	ATCTGCGCTC	TGCTGAAGCC	4500
AGTTACCTTC	GGAAAAAAGAG	TTGGTAGCTC	TTGATCCGGC	AAACAAACCA	CCGCTGGTAG	4560
CGGTGGTTT	TTTGTGCA	ACCAGCAGAT	TACCGCGAGA	AAAAAAGGAT	CTCAAGAAGA	4620
TCCTTGATC	TTTCTACGG	GGTCTGACGC	TCAGTGGAAC	AAAAACTCAC	GTAAAGGGAT	4680
TTTGGTCATG	AGATTATCAA	AAAGGATCTT	CACTAGATC	CTTTTAAATT	AAAAATGAAG	4740
TTTTAAATCA	ACTCTAAAGTA	TATATGAGTA	AACTTGGTCT	GACAGTTACC	AATGCTTAAT	4800
CAGTGAGGC	CCTATCTCAG	CGATCTGTCT	ATTTCGTTCA	TCCATAGTTG	CCTGACTCCC	4860
CGTCGTGAG	ATAACTACGA	TACGGGAGGG	CTTACCATCT	GGCCCCAGTG	CTGCAATGAT	4920
ACCGCGAGAC	CCACGCTCAC	CGGCTCAGA	TTTATCAGCA	ATAAACACGC	CAGCCGGAAG	4980
GGCCGAGCG	AGAAGTGGTC	CTGCAACTT	ATCCGCTCC	ATCCAGTCTA	TTAATTGTTG	5040
CCGGGAAGCT	AGAGTAAGTA	GTTCGCCAGT	TAATAGTTG	CGCAACGTTG	TTGCCATTGC	5100
TACAGGCATC	GTGGTGTAC	GCTCGTCGTT	TGGTATGGCT	TCATTCACT	CCGGTTCCCA	5160
ACGATCAAGG	CGAGTTACAT	GATCCCCAT	GTGTTGCAAA	AAAGCGTTA	GCTCCCTCGG	5220
TCCTCCGATC	GTGTCAGAA	GTAAGTTGGC	CGCAGTGT	TCACTCATGG	TTATGGCAGC	5280
ACTGCATAAT	TCTCTTACTG	TCATGCCATC	CGTAAGATGC	TTTCTGTGA	CTGGTGAAGTA	5340
CTCAACCAAG	TCATTCTGAG	AATAGTGTAT	CGCGCAGCG	AGTTGCTCTT	GCCCCGCGTC	5400
AATACGGGAT	AATACCGCGC	CACATAGCAG	AACTTTAAAA	GTGCTCATCA	TTGGAAAAACG	5460
TTCTTCGGGG	CGAAAACCTCT	CAAGGATCTT	ACCGCTGTTG	AGATCCAGTT	CGATGTAACC	5520
CACTCGTGCA	CCCAACTGAT	CTTCAGCATC	TTTACTTTTC	ACCAGCGTTT	CTGGGTGAGC	5580
AAAAACAGGA	AGGCAAAATG	CCGAAAAAA	GGGAATAAGG	GCGACACGGA	AATGTTGAAT	5640
ACTCATACTC	TTCCCTTTTC	AATATTATTG	AAGCATTAT	CAGGGTTATT	GTCTCATGAG	5700
CGGATACATA	TTTGAATGTA	TTTAGAAAAA	TAACAAATA	GGGGTTCCGC	GCACATTTC	5760
CCGAAAAGTG	CCACCTGACG	TCTAAGAAC	CATTATTATC	ATGACATTAA	CCTTAAAAAA	5820
TAGGCGTATC	ACGAGGCCCT	TTCGTCTCGC	CGCTTTCGGT	GATGACGGTG	AAAACCTCTG	5880
ACACATGCAG	CTCCCGGAGA	CGGTACACAGC	TTGTCTGTAA	GGGGATGCCG	GGAGCAGACA	5940
AGCCCGTCAG	GGCGCGTCAG	CGGGTGTG	CGGGTGTGCG	GGCTGGCTTA	ACTATGCGGC	6000
ATCAGAGCAG	ATTGTACTGA	GAGTGCAC				6028

Figure 10. FBdelPMOSAF Sequence

CATATGCGGT	GTGAAATACC	GCACAGATGC	GTAAGGAGAA	AATACCGCAT	CAGGCGCCAT	60
TCGCCATTCA	GGCTGCGCAA	CTGTTGGAA	GGCGATCGG	TGCGGGCTC	TTCGCTATTA	120
CGCCAGCTGG	CGAAAGGGGG	ATGTGCTGCA	AGGCGATTAA	GTTGGGTAAC	GCCAGGGTTT	180
TCCCCAGTCAC	GACGTTGTAA	AACGACGGC	AGTGAATTCC	GATTAGTCA	ATTTGTTAAA	240
GACAGGATCT	CAGTAGTCCA	GGCTTACTGC	CTGACTAAC	AATACCA	GCTAAAACCA	300
CTAGAATACG	AGCCACAATA	AATAAAGAT	TTTATTAGT	TTCCAGAGAA	AGGGGGGAAT	360
GAAAGACCCC	ACCAAATTGC	TTAGGCTGAT	AGCCGAGTA	ACGCCATT	GCAAGGCATG	420
GAAAAATACC	AAACCAAGAA	TAGAGAAGTT	CAGATCAAGG	CGGGTACAC	GAAAACAGCT	480
AACGTTGGGC	CAAACAGGAT	ATCTGCGGTG	AGCAGTTCG	GCCCCGGCC	GGGGCAAGA	540
ACAGATGGTC	ACCGCGGTT	GGCCCCGGCC	CGGGGCAAG	AACAGATGGT	CCCCAGATAT	600
GGCCCAACCC	TCAGCAGTTT	CTTAAGACCC	ATCAGATGTT	TCCAGGCTCC	CCCAAGGACC	660
TGAAATGACC	CTGTGCTTA	TTTGAATTAA	CCAATCAGCC	TGCTTCTCGC	TTCTGTTCGC	720
GCGCTTCTGC	TCCCCAGAGT	CTATAAAAGA	GCTCACAA	CCTCACTCGG	CCGCCAGTC	780
CTCCGATAGA	CTGAGTCGCC	CGGGTACCCG	TGTATCCAAT	AAATCCTTT	GCTGTGTCAT	840
CCGACTCGTG	GTCTCGCTGT	TCCCTGGGAG	GGTCTCCTCA	GAGTGAATTG	CTACCCGTCT	900
CGGGGGTCTT	TCATTGGGG	GCTCGTCCGG	GATCTGGAGA	CCCTCTGCCA	GGGACCAACCG	960
ACCCACCACC	GGGAGGTAAG	CTGGCCAAGA	TCTTATATGG	GGCACCCCCG	CCCCTGTAA	1020
ACTTCCCTGA	CCCTGACATG	ACAAGAGTTA	CTACACGCC	CTCTCTCAA	GCTCACTTAC	1080
AGGCTCTCTA	CTTAGTCCAG	CACCAAGTCT	GGAGACCTCT	GGCGCAGCC	TACCAAGAAC	1140
AACTGGACCG	ACCGGTGGTA	CCTCACCCCT	ACCGAGTCGG	CGACACAGTG	TGGTCCGCC	1200
GACACCAGAC	TAAGAACCTA	GAACCTCGCT	GGAAAGGACC	TTACACAGTC	CTGCTGACCA	1260
CCCCCACCGC	CCTCAAAGTA	GACGGCATCG	CAGCTGGAT	ACACGCC	CACGTGAAGG	1320
CTGCCGACCC	CGGGGGTGG	CCATCCTCTA	GACTGACATG	GGCGCTTCAA	CGCTCTCAA	1380
ACCCCTTAAA	AATAAGGTTA	ACCCGGGAGG	CCCCCTAATC	CCCTTAATC	TTCTGATGCT	1440
CAGAGGGGTC	AGTACTGCTT	CGCCCCGGCTC	CAGTCCTCAT	CAAGTCTATA	ATATCACCTG	1500
GGAGGTAACC	AATGGAGATC	GGGAGACGGT	ATGGGCAACT	TCTGGCAACC	ACCCCTGTG	1560
GACCTGGTGG	CCTGACCTTA	CCCCAGATT	ATGTTATGTTA	GCCCCACCATG	GACCATCTTA	1620
TTGGGGCTA	GAATATCAAT	CCCCTTTTTC	TTCTCCCCCG	GGGGCCCCCT	GTTGCTCAGG	1680
GGGCAGCAGC	CCAGGCTGTT	CCAGAGACTG	CGAAGAACCT	TTAACCTCCC	TCACCCCTCG	1740
GTGCAACACT	GCCTGGAACA	GACTCAAGCT	AGACCAAGACA	ACTCATATA	CAAATGAGGG	1800
ATTTTATGTT	TGCCCCGGGC	CCCACGGCCC	CCGAGAAATCC	AAGTCATGTG	GGGGTCCAGA	1860
CTCCTCTAC	TGTGCTTATT	GGGGCTGTGA	GACAACCGGT	AGAGCTTACT	GGAAAGCCCTC	1920
CTCATCATGG	GATTTCATCA	CAGTAAACAA	CAATCTACC	TCTGACCAGG	CTGTCCAGGT	1980
ATGCAAAGAT	AATAAGTGGT	GCAACCCCTT	AGTATTTCGG	TTTACAGACG	CCGGGAGACG	2040
GGTTACTTCC	TGGACCACAG	GACATTACTG	GGGCTTACGT	TTGTATGTC	CCGGACAAGA	2100
TCCAGGGCTT	ACATTTGGGA	TCCGACTCAG	ATACCAAAAT	CTAGGACCCC	GCGTCCAAT	2160
AGGGCCAAAC	CCCGTTCTGG	CAGACCAACA	GCCACTCTCC	AAGCCAAAC	CTGTTAAGTC	2220
GCCTTCAGTC	ACCAAACAC	CCAGTGGGAC	TCCTCTCTCC	CCTACCAAC	TTCCACCGGC	2280
GGGAACCGAA	AATAGGCTGC	TAACATTAGT	AGACGGAGCC	TACCAAGCCC	TCAACCTCAC	2340
CAGTCCTGAC	AAAACCCAAAG	AGTGTCTGGT	GTGTCTAGTA	GCGGGACCCC	CCTACTACGA	2400
AGGGGTTGCG	GTCCCTGGTA	CCTACTCCA	CCATACCTCT	GCTCCAGCCA	ACTGCTCCGT	2460
GGCCTCCCAA	CACAAGTGA	CCCTGTCCGA	AGTACCGGA	CAGGGACTCT	GCATAGGAGC	2520
AGTCCAAA	ACACATCAGG	CCCTATGTAA	TACCAACCCAG	AAAGCAGTC	GAGGGTCTTA	2580
TTATCTAGTT	GCCCCCTACAG	GTACCATGTG	GGCTTGTAGT	ACCGGGCTTA	CTCCATGCT	2640
CTCCACCACC	ATACTGAACC	TTACCACTGA	TTATTGTGTT	CTTGTGAAAC	TCTGGCAAG	2700
AGTCACCTAT	CATTCCCCA	GCTATGTTA	CGGCTGTGTT	GAGAGATCCA	ACCGACACAA	2760
AAGAGAACCG	GTGTCGTTAA	CCCTGGCCT	ATTATTGGGT	GGACTAACCA	TGGGGGAAT	2820
TGCGCTGGA	ATAGGAACAG	GGACTACTGC	TCTAATGGCC	ACTCAGCAAT	TCCAGCAGCT	2880
CCAAGCCGA	GTACAGGATG	ATCTCAGGGA	GGTTGAAAAA	TAACTCTCA	ACCTAGAAAA	2940
GTCTCTCACT	TCCCTGTCTG	AAGTTGCTCT	ACAGAATCGA	AGGGGGCTAG	ACTTGTATT	3000
TCTAAAAGAA	GGAGGGTGT	GTGCTGCTCT	AAAAGAAGAA	TGTTGCTTCT	ATGCGGACCA	3060
CACAGGACTA	GTGAGAGACA	GCATGGCCAA	ATTGAGAGAG	AGGCTTAATC	AGAGACAGAA	3120
ACTGTTGAG	TCAACTCAAG	GATGGTTGTA	GGGACTGTTT	ACAGATCCC	CTTGGTTTAC	3180
CACCTTGATA	TCTACCAATT	TGGGACCCCT	CATTGACTC	CTAATGATT	TGCTCTTCGG	3240
ACCCCTGCAATT	CTTAATCGAT	TAGTTCAATT	TGTTAAAGAC	AGGATCTCG	TAGTCAGGC	3300
TTTAGTCCTG	ACTAACAAAT	ACCACAGCT	AAAGCTTATA	GACTACAGGC	CATAGGGCGC	3360
CTAGTGTGTA	CAATTAATCA	TCGGCATAGT	ATACCGCAT	GTATAATACG	ACTCACTATA	3420
GGAGGGCCAC	CATGGCCAAG	TTGACCAGTG	CCGTTCCGGT	GCTCACCGGC	CGCGACGTCG	3480
CCGGAGCGGT	CGAGTTCTGG	ACCGACCCGG	TCGGGTTCTC	CCGGGACTTC	GTGGAGGACG	3540
ACTTCGCCGG	TGTGGTCCGG	GACGACGTGA	CCCTGTTCAT	CAGCGGGTC	CAGGACCAGG	3600
TGGTGCCTGG	CAACACCCCTG	GCCTGGGTG	GGGTGCGCGG	CCTGGACGAG	CTGTACGCCG	3660
AGTGGTCGG	GGTCGTGTCC	ACGAACCTCC	GGGACGCCCT	CGGGCCGGCC	ATGACCGAGA	3720
TCGGCGAGCA	GGCGTGGGGG	CGGGAGTTCG	CCCTGCGCGA	CCGGGCGGGC	AACTGCGTGC	3780
ACTTCGTGGC	CGAGGAGCAG	GACTGANNN	CGGACCGGTC	GACTTGTAA	CTTGTGTTATT	3840
GCAGCTTATA	ATGGTTACAA	ATAAAGCAAT	AGCATCACAA	ATTTCACAAA	TAAGCATT	3900
TTTCACTGC	ATTCTAGTTG	TGGTTTGCC	AAACTCATCA	ATGTATCTTA	TCATGTCTGG	3960
ATCCAGATCT	GGGCCCATGC	GGCCGCGGAT	CGATNNNNAC	ATGTGAGCAA	AAGGCCAGCA	4020
AAAGGCCAGG	AAACGTAAAAA	AGGCCGCGTT	GCTGGCGTT	TTCCATAGGC	TCCGCC	4080

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Figure 10. FBdelPMOSAF Sequence

2

TGACCGAGCAT	CACAAAATC	GACGCTCAAG	TCAGAGGTGG	CGAAACCGA	CAGGACTATA	4140
AAGATACCAAG	GCGTTTCCCC	CTGGAAGCTC	CCTCGTGC	TCTCCTGTT	CGACCCCTGCC	4200
GCTTACCGGA	TACCTGTCCG	CCTTCTCCC	TTCGGGAA	GTGGCGCTT	CTCAATGCTC	4260
ACGCTGTAGG	TATCTCAGTT	CGGTGTAGGT	CGTTCGCTCC	AAGCTGGCT	GTGTGCACGA	4320
ACCCCCCGTT	CAGCCCCGACC	GCTGCCCTT	ATCCGGTAAC	TATCGTCTTG	AGTCCAACCC	4380
GGTAAGACAC	GACTTATCGC	CACTGGCAGC	AGCCACTGGT	AAACAGGATTA	GCAGAGCGAG	4440
GTATCTAGGC	GGTGCTACAG	AGTTCTGAA	GTGGTGGCCT	AACTACGGCT	ACACTAGAAG	4500
GACAGTATT	GGTATCTGCG	CTCTGCTGAA	GCCAGTTACC	TTCGGAAAAA	GAGTTGGTAG	4560
CTCTTGATCC	GGCAAACAAA	CCACCCCTGG	TAGCGGTGGT	TTTTTTGTTT	GCAAGCAGCA	4620
GATTACCGCG	AGAAAAAAAG	GATCTCAAGA	AGATCCTTTG	ATCTTTCTA	CGGGGCTCTGA	4680
CGCTCAGTGG	AACGAAAAC	CACGTTAAGG	GATTTGGTC	ATGAGATTAT	AAAAAAGGAT	4740
CTTCACCTAG	ATCCTTTAA	ATTAAAAATG	AAAGTTTTAAA	TCAATCTAA	GTATATATGA	4800
GTAAAATTGG	TCTGACAGTT	ACCAATGCTT	AATCAGTGAG	GCACCTATCT	CAGCGATCTG	4860
TCTATTCGT	TCATCCATAG	TTGCTGTACT	CCCCGTCGTG	TAGATAACTA	CGATACGGGA	4920
GGGCTTACCA	TCTGGCCCCA	GTGCTCCAAT	GATACCGCGA	GACCCACGCT	CACCGGCTCC	4980
AGATTATCA	GCAATAAAC	AGCCAGCCGG	AAGGGCCGAG	CGCAGAAGTG	GTCTCTGCAAC	5040
TTTATCCGCC	TCCATCCAGT	CTATTAAATTG	TTGCCGGGAA	GCTAGAGTAA	GTAGTTCGCC	5100
AGTTAATAGT	TTGCGCAACG	TTGTTGGCAT	TGCTACAGGC	ATCGTGGTGT	CACCGCTCGTC	5160
GTGGGTATG	GCTTCATTCA	GCTCCGGTTC	CCAACGATCA	AGGCGAGTTA	CATGATCCCC	5220
CATGTTGTGC	AAAAAAGCGG	TTAGCTCCTT	CGGTCCCTCCG	ATCGTTGTCA	GAAGTAAGTT	5280
GGCCGCAGTG	TTATCACTCA	TGGTTATGGC	AGCACTGCAT	AATTCTCTTA	CTGTCATGCC	5340
ATCCGTAAGA	TGCTTTCTG	TGACTGGTGA	GTACTCAACC	AAGTCATTCT	GAGAATAGTG	5400
TATGCCCGA	CCGAGTTGCT	TTGCCCGGC	GTOAATACGG	GATAATACCG	CGCCACATAG	5460
CAGAACCTTA	AAAGTGCTCA	TCATTGGAAA	ACGTCTTCG	GGGCGAAAAC	TCTCAAGGAT	5520
CTTACCGCTG	TTGAGATCCA	TTTCGATGTA	ACCCACTCGT	GCACCCAAC	GATCTTCAGC	5580
ATCTTTACT	TTCACCAAGCG	TTTCTGGGT	AGCAAAAACA	GGAAAGGCAA	ATGCCGCAA	5640
AAAGGGAATA	AGGGCGACAC	GGAAATGTTG	AATACTCATA	CTCTTCTTT	TTCAATATTA	5700
TTGAAGCATT	TATCAGGGTT	ATTGTCTCAT	GAGCGGATAC	ATATTGAAAT	GTATTAGAA	5760
AAATAAACAA	ATAGGGGTT	CGCGCACATT	TCCCCGAAAA	GTGCCACCTG	ACGCTAAGA	5820
AACCATTATT	ATCATGACAT	TAACCTATAA	AAATAGGCCT	ATCACGAGGC	CCTTCGTCT	5880
CGCGCGTTTC	GGTGATGACG	GTGAAAACCT	CTGACACATG	CAGCTCCCCG	AGACGGTCAC	5940
AGCTTGTCTG	TAAGCGGATG	CCGGGAGCAG	ACAAGCCCGT	CAGGGCGCGT	CAGCGGGTGT	6000
TGGCGGGTGT	CGGGGCTGGC	TTAACTATGC	GGCATCAGAG	CAGATTGTAC	TGAGAGTGCA	6060
C						6061

Figure 11. FBdelPGASAF Sequence

CATATGCGGT	GTGAAATACC	GCACAGATGC	GTAAGGAGAA	AATACCGCAT	CAGGCGCCAT	60
TCGCCATTCA	GGCTGCGAA	CTGTTGGAA	GGCGGATCGG	TGCGGGCTC	TTCGCTATTA	120
CGCCAGCTGG	CGAAAGGGGG	ATGTGCTGCA	AGGGGATTAA	TTTGGGTAAC	GCCAGGGTTT	180
TCCCACTCAC	GACGTTGTA	AACGACGGG	AGTGAATTCC	GATTAGTCA	ATTGGTTAAA	240
GACAGGATCT	CAGTACTCCA	GGCTTACTGC	CTGACTCAAC	AATACCCACA	GCTAAAACCA	300
CTAGAATACG	AGCCACAATA	AATAAAGAT	TTTATTTAGT	TTCCAGAAAA	AGGGGGAAAT	360
GAAAGACCCC	ACCAAATTG	TTAGGCTGAT	AGCCGAGTA	ACGCACTT	GCAAGGCATG	420
AAAAAATACC	AAACCAAGAA	TAGAGAAGTT	CAGATCAAGG	GCGGGTACAC	GAAAACAGCT	480
AACGTTGGGC	CAAACAGGAT	ATCTGCGGTG	AGCAGTTTCG	GCCCCGGCCC	GGGGCAAGA	540
ACAGATGGTC	ACCGCGGTC	GGCCCCGGCC	CGGGGCAAG	AACAGATGGT	CCCCAGATAT	600
GGCCCAACCC	TCAGCAGTTT	CTTAAGACCC	ATCAGATGTT	TCCAGGCTCC	CCCAAGGACC	660
TGAAATGACC	CTGTGCTTAA	TTTGAATTAA	CCAATCAGCC	TGCTTCTCG	TTCTGTTCGC	720
GCGCTTCTGC	TCATCCAGGCT	CTATAAAGA	GCTACAAC	CCTCACTCGG	CGGCCAGTC	780
CTCCGATAGA	CTGAGTCGCC	CGGGTACCCG	TGTATCCAAT	AAATCCTCTT	GCTGTTGCAT	840
CCGACTCGTG	GTCTCGCTGT	TCCTTGGAG	GGTCTCTCA	GAGTGTGAA	CTACCCGTCT	900
CGGGGGTCTT	TCATTGGGG	GCTCGTCCGG	GATCTGGAGA	CCCTCTCCCA	GGGACCCACCG	960
ACCCACCACC	GGGAGGTAAG	CTGGCCAAGA	TCCCTAAGGT	ACTCGGGTCA	GACAATGGCC	1020
CGGCCCTTGT	TGCTCAGGTA	AGTCAGGGAC	TGGCCACTCA	ACTGGGGATA	AATTGGAAGT	1080
TACATTGTGC	GTATAGACCC	CAGAGCTCAG	GTCAGGTAGA	AAGAATGAAC	AGAACAAATTA	1140
AAGAGACCTT	GACCAAATT	GCCTTAGAGA	CCGGTGGAAA	AGACTGGGTG	ACCCCTCTTC	1200
CCTTAGCGCT	GCTTAGGGCC	AGGAATACCC	CTGGCGGTT	TGGTTAACT	CCTTATGAAA	1260
TTCTCTATGG	AGGACCACCC	CCCCATACTTG	AGTCTGGAGA	AACTTGGGT	CCCGATGATA	1320
GATTTCTCCC	TGTCTTATTT	ACTCACTTAA	AGGCTTTAGA	AATTGTAAGG	ACCCAAATCT	1380
GGGACCAGAT	CAAAGAGGTG	TATAAGCCTG	GTACCGTAAC	AATCCCTCAC	CCGTTCCAGG	1440
TCGGGGATCA	AGTGCTTGTG	AGACGCCATC	GACCCAGCAG	CCTTGAGCCT	CGGTGGAAAG	1500
GCCCCATACCT	GGTGTGCTG	ACTACCCCGA	CCGGGGTAAA	AGTCGATGGT	ATTGCTGCCT	1560
GGGTCCATGC	TTCTCACCTC	AAACCTGCAC	CACCTTCGGC	ACCAGATGAG	TCCTGGGAGC	1620
TGGAAAAGAC	TGATCATCCT	CTTAAGCTG	GTATTGGCG	GGCGCGGGAC	GAGTCTGCAA	1680
AATAAGAAC	CCCACCAGCC	CATGACCTC	ACTTGGCAGG	TACTGCCCCA	AACCTGGAGAC	1740
GTTGCTCGGG	ATACAAAGG	AGTCCAGCCC	CCTTGGACTT	GGTGGCCCAC	ACTTAAACCT	1800
GATGTATGTG	CCTTGGCGGC	TAGTCTTGAG	TCCTGGATA	TCCCGGGAAC	CGATGTCTCG	1860
TCCTCTAAC	GAGTCAGACC	TCCGGACTCA	GACTATACTG	CCGCTTATAAA	GAAATCACC	1920
TGGGGAGCCA	TAGGGTGCAG	CTACCCCTGG	GCTAGGACTA	GAATGGCAAG	CTCTACCTTC	1980
TACGTATGTC	CCCCGGATGG	CCGGACCCCT	TCAGAAGCTA	GAAGGTGCGG	GGGGCTAGAA	2040
TCCCTTAACT	GTAAAGAATG	GGATTGTGAG	ACCACGGGAA	CCGGGTATTG	GCTATCTAAA	2100
TCCTCAAAAG	ACCTCATAAC	TGTAAAATGG	GACCAAAATA	GCGAATGGAC	TCAAAAATTT	2160
CAACAGTGT	ACCAGACCGG	CTGGTGTAA	CCCCTTAAAA	TAGATTTCAC	AGACAAAGGA	2220
AAATTATCCA	AGGACTGGAT	AACGGGAAAA	ACCTGGGGAT	TAAGATTCTA	TGTGCTGGA	2280
CATCCAGGCG	TACAGTTCAC	CATTGCTTA	AAAATCACCA	ACATGCCAGC	TGTGGCAGTA	2340
GGTCCTGACC	TCGTCCTTGT	GGAACAAAGG	CCTCTCTAGAA	CGTCCCTCGC	TCTCCCACCT	2400
CCTCTTCCCC	CAAGGGAAAGC	GCCACCGCCA	TCTCTCCCCG	ACTCTAAC	CACAGCCCTG	2460
GCGACTAGTG	CACAAACTCC	CACGGTGA	AAAACAATTG	TTACCTTAA	CACTCCGCCT	2520
CCCACCAACAG	CGGACAGACT	TTTTGATCTT	GTGCAGGGGG	CCTTCCTAAC	CTTAAATGCT	2580
ACCAACCCAG	GGGCCACTGA	GTCTTGCTGG	CTTTGTTGG	CCATGGGCC	CCCTTATTAT	2640
GAAGCAATAG	CCTCATCAGG	AGAGGTGCC	TACTCCACCG	ACCTTGACCG	GTGCCGCTGG	2700
GGGACCAAG	GAAAGCTCAC	CCTCACTGAG	GTCTCAGGAC	ACGGGTTGTG	CATAGGAAAG	2760
GTCCTTTTAA	CCCATCAGCA	TCTCTGCAAT	CAGACCTAT	CCATCAATT	CTCCGGAGAC	2820
CATCAGTATC	TGCTCCCTC	CAACCATAGC	TGGTGGGCTT	CGAGCACTGG	CCTCACCCCT	2880
TGCTCTCCA	CCTCAGTTTT	TAATCAGACT	AGAGATTCT	TTATCCAGGT	CCAGCTGATT	2940
CCTCGCATCT	ATTACTATCC	TGAAGAAGTT	TTGTTACAGG	CCTATGACAA	TTCTCACCCCC	3000
AGGACTAAAA	GAGAGGCTGT	CTCACTTACC	CTAGCTGTTT	TACTGGGGTT	GGGAATCAGC	3060
GCGGGAAATAG	GTACTGGTTG	AACTGCCTA	ATTTAAAGGAC	CTATAGACCT	CCAGCAAGGC	3120
CTGACAAGCC	TCCAGATCGC	CATAGATGCT	GACCTCCGGG	CCCTCCAAGA	CTCAGTCAGC	3180
AAGTTAGAGG	ACTCACTGAC	TTCCCTGTCC	GAGGTAGTGC	TCCAAAATAG	GAGAGGCCTT	3240
GACTTGTGT	TTCTAAAAGA	AGGTGGCC	TGTGGGGCCC	TAAAGGAAGA	GTGCTGTTTT	3300
TACATAGACC	ACTCAGGTG	AGTACGGGAC	TCCATGAAAA	AACCTAACAG	AAAACCTGGAT	3360
AAAAGACAGT	TAGAGCGCCA	GAAAAGC	AACTGGTATG	AGGAGTGGTT	CAATAACTCC	3420
CCTTGGTTCA	CTACCCCTGCT	ATCAACCATC	GCTGGGGCCC	TATTACTCT	CCTTCCTGTTG	3480
CTCATCCTCG	GGCCATGCA	CATCAATCGA	TTAGTTCAAT	TTGTTAAAGA	CAGGATCTCA	3540
GTAGTCAGG	CTTTAGTCTC	GACTCAACAA	TACCACCA	TAAAGCCTAT	AGAGTACGAG	3600
CCATAGGGCG	CCTAGTGTG	ACAATTAAATC	ATCGCAGATAG	TATACGGCAT	AGTATAATAC	3660
GACTCACTAT	AGGAGGGCCA	CCATGGCCAA	GTTGACCA	GCCGTTCCGG	TGCTCACCGC	3720
GCGCGACGTC	GGCCGGAGCGG	TCGAGTCTG	GACCGACCGG	CTCGGGTTCT	CCCGGGACTT	3780
CGTGGAGGAC	GACTTCGCCG	GTGTGGTCCG	GGACGACGTG	ACCCCTGTTCA	TCAGGGCGGT	3840
CCAGGACCAG	GTGGTGCCG	ACAACACCT	GGCCTGGGTG	TGGGTGCCG	GCCTGGACGA	3900
GCTGTACGCC	GAGTGGTCGG	AGGTCGTGTC	CACGAAC	CGGGACGCC	CCGGGGCCGC	3960
CATGACCGAG	ATCCGCGAGC	AGCCGTGGGG	GGGGAGTTC	GCCCTGCG	ACCCGGCCGG	4020
CAACTGCGT	CACTTCGTTG	CCGAGGAGCA	GGACTGANN	NCGGACCGGT	CGACTTGT	4080

Figure 11. FBdelPGASAF Sequence

ACTTGTAT	TGCAGCTTAT	AATGGTTACA	AATAAAGCAA	TAGCATCACAA	AATTTCACAA	4140
ATAAACGATT	TTTTTCACTG	CATTCTAGTT	GTGGTTGTC	CAAACATC	AATGTATCTT	4200
ATCATGTCTG	GATCCAGATC	TGGGCCATG	CGGCCGCGGA	TCGATNNNNA	CATGTGAGCA	4260
AAAGGCCAGC	AAAAGGCCAG	GAACCGTAAA	AAGGCCGCGT	TGCTGGCGTT	TTCCCATAGG	4320
CTCCGCCCGC	CTGACGAGCA	TCACAAAAAT	CGACGCTCAA	GTCAGAGGTG	GCGAAACCCG	4380
ACAGGACTAT	AAAGATACCA	GGCGTTTCCC	CCTGGAAGCT	CCCTCGTGC	CTCTCCGTGTT	4440
CCGACCCCTGC	CGCTTACGGG	ATACCTGTCC	GCTTTCTCC	CTTCGGGAAG	CCTGGCGCTT	4500
TCTCAATGCT	CACGCTGTAG	GTATCTCAGT	TCGGGTGAGG	TGCTTCGCTC	CAAGCTGGGC	4560
TGTGTGCACG	AACCCCCCGT	TCAGCCGAC	CGCTGCGCCT	TATCCGTAA	CTATCGTCTT	4620
GAGTCCAACC	CGGTAAGACA	CGACTTATCG	CCACTGGCAG	CAGCCACTGG	TAACAGGATT	4680
AGCAGAGCGA	GGTATGTAGG	CGGTGCTACA	GAGTTCTGTA	AGTGGTGGCC	TAACTACGGC	4740
TACACTAGAA	GGACAGTATT	TGGTATCTGC	GCTCTGCTGA	AGCCAGTTAC	CTTCGGAAAA	4800
AGAGTTGGTA	GCTCTTGATC	CGGAAACAA	ACCACCGCTG	GTAGCGGTGG	TTTTTTGTT	4860
TGCAAGCAGC	AGATTACGCC	CAGAAAAAAA	GGATCTCAAG	AAGATCCTT	GATCTTTCT	4920
ACGGGGTCTG	ACGCTCAGTG	GAACGAAAAC	TCACGTTAAG	GGATTTGGT	CATGAGATT	4980
TCAAAAGGA	TCTTCACCTA	GATCCTTTA	AATTAAAAAT	GAAGTTTAA	ATCAATCTAA	5040
AGTATATATG	AGTAAACTTG	GTCTGACAGT	TACCAATGCT	TAATCAGTA	GGCACCTATC	5100
TCAGCGATCT	GTCTATTTCG	TTCATCCATA	GTGCGCTGAC	TCCCCGTCGT	GTAGATAACT	5160
ACGATACGGG	AGGGCTTACC	ATCTGGCCCC	AGTGTGCAA	TGATACCGCG	AGACCCACGC	5220
TCACCCGCTC	CAGATTATTC	AGCAATAAAC	CAGCCAGCCG	GAAGGGCCGA	GCGCAGAACT	5280
GGTCTGCAA	CTTTATCCGC	CTCCATCCAG	TCTATTAAATT	GTGCGCGGA	AGCTAGAGTA	5340
AGTAGTTCGC	CGTTAACATAG	TTTGCACAA	GTGTTGCCA	TTGCTACAGG	CATCGTGGTG	5400
TCACGCTCGT	CGTTTGGTAT	GGCTTCATT	AGCTCCGGTT	CCCAACGATC	AAGGGAGGTT	5460
ACATGATCCC	CCATGTTGTG	CAAAAAAGCG	GTAGCTCCT	TCGGTCTCC	GATCGTTGTC	5520
AGAAGTAAGT	TGGCCGCAGT	GTTATCACTC	ATGGTTATGG	CAGCACTGCA	TAATCTCTT	5580
ACTGTCATGC	CATCCGTAAG	ATGCTTTCT	GTGACTGGTG	AGTACTCAAC	CAAGTCATTC	5640
TGAGAATAGT	GTATGCGGCG	ACCGAGTTGC	TCTGCCCGG	CGTCAATACG	GGATAATACC	5700
GCGCCACATA	GCAGAACTTT	AAAAGTGCTC	ATCATTGGAA	AACGTTCTTC	GGGGCGAAAA	5760
CTCTCAAGGA	TCTTACCGCT	GTTGAGATCC	AGTCGATGT	AACCCACTCG	TGCACCCAAAC	5820
TGATCTTCAG	CATCTTTAC	TTTCACCGAC	GTTCCTGGGT	GAGCAAAAC	AGGAAGGCAA	5880
AATGCCGCAA	AAAAGGGAAT	AAAGGGCACA	CGGAAATGTT	GAATACTCAT	ACTCTCCCTT	5940
TTTCAATATT	ATTGAAGCAT	TTATCAGGGT	TATTGTCTCA	TGAGCGGATA	CATATTGAA	6000
TGTATTAGA	AAAATAAAC	AATAGGGTT	CCGCGCACAT	TTCCCCGAAA	AGTGCCACCT	6060
GACGCTAAG	AAACCATTAT	TATCATGACA	TTAACCTATA	AAAATAGGCG	TATCACGAGG	6120
CCCTTTCGTC	TCGCGCGTT	CGGTGATGAC	GGTAAAAC	TCTGACACAT	GCAGCTCCCG	6180
GAGACGGTCA	CAGTTGTCT	GTAAGGGAT	GCCGGGAGCA	GACAAGCCCG	TCAGGGCGCG	6240
TCAGCGGGTG	TTGGCGGGTG	TCGGGGCTGG	CTTAACATAG	CGGCATCAGA	GCAGATTGTA	6300
CTGAGAGTGC	AC					6312

Figure 12. FBdelPRDSAF Sequence

CATATGCGGT	GTGAAATACC	GCACAGATGC	GTAAGGAGAA	AATACCGCAT	CAGGGGCCAT	60
TCGCCATTCA	GGCTGCGCAA	CTGTTGGAA	GGCGGATCGG	TGCGGGCTC	TTCGCTATTAA	120
CGCCAGCTGG	CGAAAGGGGG	ATGTGCTGCA	AGGCGATTAA	GTTGGGTAAC	GCCAGGGTTT	180
TCCCAAGTCAC	ACAGGTGTA	AACGACGGG	AGTGAATTCC	GATTAGTCA	ATTGGTTAAA	240
GACAGGATCT	CACTAGTCCA	GGCTTTAGTC	CTGACTCAAC	AATACCAACCA	GCTAAAACCA	300
CTAGAATACG	AGCCACAATA	AATAAAAGAT	TTTATTTAGT	TTCCAGAAAA	AGGGGGAAAT	360
GAAAGACCCC	ACCAAATTGC	TTAGCCTGAT	AGCCGCAGTA	ACGCCATTTC	GCAAGGCATG	420
GAAAATACC	AAACCAAGAA	TAGAGAAGTT	CAGATCAAGG	GCGGGTACAC	GAAAACAGCT	480
AACGTTGGGC	CAAAACAGGAT	ATCTGCGGTG	AGCAGTTTCG	GCCCCGGCCC	GGGGCCAAGA	540
ACAGATGGTC	ACCGCGGTC	GGCCCCGGCC	CGGGGCCAAG	AACAGATGGT	CCCCAGATAT	600
GGCCCAACCC	TCAGCAGTTT	CTTAAGACCC	ATCAGATGTT	TCCAGGCTCC	CCCAAGGACC	660
TGAAATGACC	CTGTGCTTAA	TTTGAATTAA	CCAATCAGCC	TGCTTCTCCG	TTCTGTTCGC	720
GCGCTCTGC	CTTCCCGAGCT	CTATAAAAGA	GCTCACAAAC	CCTCACTCGG	CGGCCAGTC	780
CTCCGATAGA	CTGAGTCGCG	CGGGTACCCG	TGTATCCAAC	AAATCTCTT	GCTGTTGCAT	840
CCGACTCGTG	GTCTCGCTGT	TCCTTGGGAG	GGTCTCTCA	GAGTGTATTGA	CTACCCGTCT	900
CGGGGGTCTT	TCATTGGGG	GCTCGTCCGG	GATCTGGAGA	CCCCTGCCCA	GGGACCACCG	960
ACCCACCACC	GGGAGGTAAG	CTGGCCAAGA	TCCCCCGGGC	TGCAAGGATT	TATGAAATCC	1020
TTTATGGGGG	ACCCCCCCCCT	TTGTCACACCT	TGCTCAATTTC	CTTCTCCCCC	TCCGATCCTA	1080
AGACTGATT	ACAAGCCCGA	CTAAAAGGGG	TGCAAGGCGT	GCAGGCCAA	ATCTGGACAC	1140
CCCTGGCCGA	ATGTGACCGG	CCAGGACATC	CACAAACTAG	CCACCCATT	CAGGTGGGAG	1200
ACTCCGTGTA	CGTCCGGCGG	CACCGCTCTC	AAGGATTGGA	GCCTCGTTGG	AAGGGACCTT	1260
ACATCGTCT	GCTGACCCAG	CCCACCGCCA	AAAGGTTGA	CGGGGATGCC	GCCTGGATTTC	1320
ACGCATCGCA	CGCCAAGGCA	GCCCCAAAAA	CCCCTGGACC	AGAAACTCCC	AAAACCTGGA	1380
AGCTCCGCGG	TCGGGAGAAC	CCTCTTAAGA	TAAGACTCTC	CCGTGTCTGA	CTGCTAATCC	1440
ACCTTGTCCC	TGACTAACC	CAAAATGAAA	CTCCCAACAG	GAATGGTCAT	TTTATGTAGC	1500
CTAATAATAG	TTCGGGCGAGG	GTGGACGAC	CCCCGCAAGG	CTATCGCATT	AGTACAAAAAA	1560
CAACATGGTA	AACCATCGCA	ATGCAGCGGA	GGGCAGGTAT	CCGAGGCC	ACCGAACTCC	1620
ATCCAACAGG	TAACTTGGCC	AGGCAAGACG	GCCTACTTAA	TGACCAACCA	AAAATGGAAA	1680
TGCAGAGTC	CTCCAAAAAT	CTCACCTAGC	GGGGGAGAAC	TCCAGAACGT	CCCCGTAAAC	1740
ACTTTCCAGG	ACTCGATGCA	CAGTTCTGT	TATACTGAAT	ACCGGCAATG	CAGGCCAATT	1800
AATAAGACAT	ACTACACCGC	CACCTTGCTT	AAAATACGGT	CTGGGAGCCT	CAACGAGGTA	1860
CAGATATTAC	AAAACCCCAA	TCAGCTCTTA	CAGTCCCCCTT	GTAGGGCTC	TATAAATCAG	1920
CCCGTTTGCT	GGAGTGCCAC	AGCCCCCATC	CATATCTCCG	ATGGTGGAGG	ACCCCTCGAT	1980
ACTAAGAGAG	TGTTGAGACT	CCAAAAAAAGG	CTAGAACAAA	TTCAATAAGG	TATGACTCCT	2040
GAACCTCAAT	ACCAACCCCTT	AGCCCTGCC	AAAGTCAGAG	ATGACCTTAG	CCTTGATGCA	2100
CGGACTTTG	ATATCCTGAA	TACCACTTTT	AGGTTACTCC	AGATGTCAA	TTTGTACCTT	2160
GCCCAGATT	GTGGCTCTG	TTTAAACTA	GGTACCCCTA	CCCCCTTGC	GATAACCCACT	2220
CCCTCTTAA	CCTACTCCCT	AGCAGACTCC	CTAGCGAATG	CCTCCGTCA	GATTATACCT	2280
CCCTCTTGG	TTCAACCGAT	GCAGTTCTCC	AACCTGCTCT	GTTTATCTC	CCCTTTCATT	2340
AACGATACCG	ACAAAATAGA	CTTAGGTGCA	GTACCCCTTA	CTAACTGAC	CTCTGTAGCC	2400
AATGTCAGTA	GTCCCTTATG	TGCCCTAAC	GGGTCACT	TCCCTGTGG	AAATAACATG	2460
GCATACACCT	ATTTACCCCA	AAACTGACG	AGACTTTGCG	TCCAAGCCT	CCTCCCTCCCC	2520
GACATTGACA	TCAACCCGGG	GGATGAGCCA	GTCCCCATTG	CTGCCATTGA	TCATTATATA	2580
CATAGACCTA	AACGAGCTGT	ACAGTTCATC	CCTTTACTAG	CTGGACTGGG	AATCACCGCA	2640
GCATTCTACCA	CCGGAGCTAC	AGGCCTAGGT	GTCTCCGTCA	CCCAGTATAC	AAAATTATCC	2700
CATCAGTTAA	TATCTGATGT	CCAAGCTTAA	TCCGGTACCA	TACAAGATT	ACAAGACCA	2760
GTAGACTCGT	TAGCTGAAGT	AGTTCTCAA	AAATAGGAGG	GACTGGACT	ACTAACGGCA	2820
GAACAAGGAG	GAATTGTTT	AGCCTTACAA	AAAAATGCT	GTTTTATGTC	TAACAAGTCA	2880
GGAATTGTA	GAACAAAAAT	AAAAGCCTA	CAAGAAGAAT	TACAAAACG	CAGGGAAAGC	2940
CTGGCAACCA	ACCCCTCTG	GACCGGGCTG	CAGGGTTTC	TTCCGTACCT	CCTACCTCTC	3000
CTGGGACCCC	TACTCACCC	CCTACTCATA	CTAACCATTG	GGCATGCGT	TTTCAGTCGC	3060
CTCATGGCCT	TCATTAATGA	TAGACTTAAT	GTTGTACATG	CCATGGTGCT	GGCCCAGCAA	3120
TACCAAGC	TCAAAGCTG	GGAGGAAGCT	CAGGATTGAG	GCGCCTAGTG	TTGACAAATT	3180
ATCATCGCA	TAGTATACGG	CATAGTATAA	TACGACTCAC	TATAGGAGGG	CCACCATGGC	3240
CAAGTTGACC	AGTGCCGTT	CGGTGCTCAC	CGGCCGCGAC	GTCGCCGGAG	CGGTGAGTT	3300
CTGGACCGAC	CGGCTCGGGT	TCTCCCGGGG	CTTCGTGGAG	GACGACTTCG	CGGGTGTGGT	3360
CCGGGACGAC	GTGACCCCTG	TCATCAGCGC	GGTCCAGGAC	CAGGTGGTGC	CGGACAAACAC	3420
CCTGGGCTGG	GTGTGGGTG	CGGGCCTGGA	CGAGCTGTAC	GCCGAGTGGT	CGGAGGTCGT	3480
GTCCACGAA	TTCCGGGACG	CCTCCGGGCG	GGCCTGACCC	GAGATCGGCG	AGCAGCCGTG	3540
GGGGCGGGAG	TTCGCCCTG	GCGACCCGGC	CGGCAACTGC	GTGCACCTCG	TGGCCGAGGA	3600
GCAGGACTGA	NNNNCCGACC	GGTCGACTTG	TTAACCTTGTT	TATITGCAGCT	TATAATGGTT	3660
ACAAATAAAG	CAATAGCATC	ACAAATTTC	CAAATAAAGC	ATTTTTTCA	CTGCATTCTA	3720
GTTGTTGTTT	GTCCAAACTC	ATCAATGAT	CTTATCATGT	CTGGATCCAG	ATCTGGGCC	3780
ATGCGCCGCG	GGATCGATNN	NNACATGTGA	GCAAAGGCC	AGCAAAGGC	CAGGAACCGT	3840
AAAAAGGCGC	CGTTGCTGGC	GTTTTCCAT	AGGCTCCGCC	CCCCTGACCA	GCATCACAAA	3900
AATCGACGCT	CAAGTCAGAG	GTGGCGAAC	CCGACAGGAC	TATAAAGATA	CCAGGGCTTT	3960
CCCCCTGGAA	GCTCCCTCGT	GCGCTCTCC	GTTCCGACCC	TGCCGCTTAC	CGGATACCTG	4020
TCCGCCCTTC	TCCCTTCGGG	AAAGCGTGGCG	CTTCTCTCAAT	GCTCACGCTG	TAGGTATCTC	4080

Figure 12. FBdelPRDSAF Sequence

2

AGTTCCGTGT	AGGTCTGTTCG	CTCCAAGCTG	GGCTGTGTGC	ACGAACCCCC	CGTTCAGCCC	4140
GACCGCTGCG	CCTTATCCGG	TAACTATCGT	CTTGAGTCCA	ACCCGGTAAG	ACACGACTTA	4200
TCGCCACTGG	CAGCAGCCAC	TGGTAACAGG	ATTAGCAGAG	CGAGGTATGT	AGGCCGTGCT	4260
ACAGAGTTCT	TGAAGTGGTG	GCCTAACTAC	GGCTACACTA	GAAGGACAGT	ATTTGGTATC	4320
TGGCCTCTGC	TGAAGGCCAGT	TACCTTCGGA	AAAAGAGTTG	GTAGCTCTTG	ATCCGGAAA	4380
CAAACCACCG	CTGGTAGCGG	TGGTTTTTT	GTTTGCAAGC	ACGAGATTAC	GCGCAGAAAA	4440
AAAGGATCTC	AAGAAGATCC	TTTGATCTTT	TCTACGGGGT	CTGACGCTCA	GTGGAACGAA	4500
AACTCACGTT	AAGGGATTTT	GGTCATGAGA	TTATCAAAAAT	GGATCTTCAC	CTAGATCCTT	4560
TTAAATTAAA	AATGAAGTTT	TAAATCAATC	TAAGTATAT	ATGAGTAAAC	TTGGTCTGAC	4620
AGTTACCAAT	GCTTAATCAG	TGAGGCACCT	ATCTCAGCGA	TCTGTCATT	TCGTCATCC	4680
ATAGTTGCCT	GACTCCCCGT	CGTGTAGATA	ACTACGATAC	GGGAGGGCTT	ACCATCTGGC	4740
CCCGATGCTG	CAATGATACC	GCGAGACCCA	CGCTCACCGG	CTCCAGATT	ATCAGCAATA	4800
AACCAGCCAG	CCGGAAGGGC	CGAGCGCAGA	AGTGGTCCCTG	CAACTTTATC	CGCCTCCATC	4860
CAGTCTATTA	ATGTGTCGG	GGAAAGCTAGA	GTAAGTAGTT	CGCCACTTAA	TAGTTTGC	4920
AACGTTGTTG	CCATTGCTAC	AGGCATCGTG	GTGTCACGGCT	CGTCGTTTGG	TATGGCTTCA	4980
TTCAGCTCCG	GTTCCTAACCG	ATCAAGGCGA	GTTACATGAT	CCCCCATGTT	GTGCAAAAAA	5040
GCGGTTAGCT	CCTTCGGTCC	TCCGATCGTT	GTCAAGAGTA	AGTTGGCCGC	AGTGTATCA	5100
CTCATGGTTA	TGGCAGCACT	GCATAATTCT	CTTACTGTCA	TGCCATCCGT	AAGATGTTT	5160
TCTGTGACTG	GTCAGTACTC	AACCAAGTCA	TTCTGAGAAT	AGTGTATGCG	GCGACCCAGT	5220
TGCTCTTGC	CGCGCGTCAAT	ACGGGATAAT	ACCGCGCCAC	ATAGCAGAAC	TTTAAAAGTG	5280
CTCATCATTG	GAAAACGTT	TTCGGGCGA	AAACTCTCAA	GGATCTTAC	GCTGTTGAGA	5340
TCCAGTTCGA	TGTAACCCAC	TCGTGCACCC	AACTGATCTT	CAGCATCTT	TACTTTCA	5400
AGCGTTCTG	GGTGAGCAA	ACAGGAAGG	AAAATGCCG	CAAAAGGG	AATAAGGGCG	5460
ACACGGAAAT	GTGAAACT	CATACTCTTC	CTTTTTCAAT	ATTATTGAAG	CATTATCAG	5520
GGTTATTGTC	TCATGAGCGG	ATACATATT	GAATGTATT	AGAAAATAA	ACAAATAGGG	5580
GTTCCCGCGCA	CATTTCGGCG	AAAAGTCCA	CCTGACGTCT	AAGAAAACAT	TATTATCATG	5640
ACATTAACCT	ATAAAAAATAG	GCGTATCACG	AGGCCCTTTC	GTCTCGCGG	TTTCGGTGAT	5700
GACGGTGAAA	ACCTCTGACA	CATGCAGCTC	CCGGAGACGG	TCACAGCTG	TCTGTAAGCG	5760
GATGCCGGGA	GCAGACAAGC	CCGTCAGGGC	GCCTCAGCGG	GTGTTGGCGG	GTGTCGGGCC	5820
TGGCTTAACT	ATGCGGCATC	AGAGCAGATT	GTACTGAGAG	TGCAC		5865

Figure 13. hCMV10A1 Sequence

AGATCTCCCG	ATCCCCATAG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	60
AGCCAGTATC	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGC	GAGCAAAATT	120
TAAGCTACAA	CAAGGCAAGG	CTTGACCGAC	AATTCATGA	AGAATCTGCT	TAGGGTTAGG	180
CGTTTGC	TGCTTCGCA	TGTACGGGAC	AGATATAACCC	GTTGACATTG	ATTATTGACT	240
AGTTTAAAT	AGTAAATCAAT	TACGGGTC	TTAGTTCAT	GCCCATATAT	GGAGTTCCGC	300
GTTACATAAC	TTACGGTAA	TGGCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	360
ACGTCAATAA	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	420
TGGGTGGACT	ATTTACGGTA	AACTGCCAC	TTGGCAGTAC	ATCAAGTGT	TCATATGCCA	480
AGTACGCC	CTATTGACGT	CAATGACGGT	AAATGGCCCG	CCTGGCATT	TGCCAGTAC	540
ATGACCTTAT	GGGACTTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	600
ATGGTGATGC	GGTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTGA	CTCACGGGA	660
TTTCCAAGTC	TCCACCCCAT	TGACGTCAAT	GGGAGTTGT	TTTGGCACCA	AAATCAACGG	720
GACTTTCCA	AATGTGCTAA	CAACTCCGCC	CCATTGACGC	AAATGGCCG	TAGGGGTGTA	780
CGGTGGGAGG	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTAACIG	840
GCTTATCGAA	ATGTCGACTG	AGAACTTCAG	GGTGAGTTG	GGGACCCCTG	ATTGTTCTTT	900
CTTTTCGCT	ATTGTAAAAT	TCATGTTATA	TGGAGGGGC	AAAGTTTCA	GGGTGTTGTT	960
AGAATGGGA	AGATGTCCCT	TGTATCACCA	TGGACCCCTCA	TGATAATT	TTTCTTTCA	1020
CTTTTCACTC	TGTTGACAAAC	CATTGCTCTC	TCTTATTTC	TTTCATT	CTGTAACTTT	1080
TTCGTTAAAC	TTAGCTTGC	ATTTGTAACG	AATTTTAAA	TTCACTTTG	TTTATTGTC	1140
AGATTGTAAG	TACTTTCTC	AATCACTTT	TTTCAAGGC	AATCAGGGTA	TATTATATTG	1200
TACTTCAGCA	CAGTTTAGA	GAACATTGT	TATAATTAA	TGATAAGGTA	GAATATTCT	1260
GCATATAAAAT	TCTGGCTGGC	GTGGAATAT	TCTTATTGGT	GAAGAACAACT	ACATCCTGGT	1320
CATCATCCTG	CTTTCTCTT	TATGGTTACA	ATGATATACA	CTGTTTGAGA	TGAGGATAAA	1380
ATACTCTGAG	TCCAAACCGG	GCCCCCTCTG	TAACCATGTT	CATGCTTCT	TCTTTTCCCT	1440
ACAGCTCCGT	GGCAACGTGC	TGGTTGTTGT	GCTGCTCAT	CATTTGGCA	AGGATCGGCC	1500
GGAACAGCAT	CAGGACCGAC	ATGGAAGGTC	CAGCGTTCTC	AAAACCCCTT	AAAGATAAGA	1560
TTAACCCGTG	GAAGTCTTAA	ATGGTCATGG	GGGTCATTT	AAGAGTAGGG	ATGGCAGAGA	1620
GCCCCCATCA	GGTCTTAAAT	GTAAACCTGGA	GAGTCACAA	CCTGATGACT	GGGCGTACCG	1680
CCAATGCCAC	CTCCCCCTTA	GGAACTGTAC	AAAGATGCC	CCCAAGGATA	TATTGATC	1740
TATGTGATCT	GGTCGGAGAA	GAGTGGGACC	CTTCAGACCA	GGAAACCAT	GTGGGTATG	1800
GCTGCAAATA	CCCCGGAGGG	AGAAACCGGA	CCCCGACTTT	TGACTTTAC	GTGTGCCCTG	1860
GGCATACCGT	AAAATCGGGG	TGTGGGGGC	CAAGAGAGGG	CTACTGTGGT	GAATGGGGTT	1920
GTGAAACAC	CGGACAGGTC	TACTGGAAGC	CCACATCATC	ATGGGACCTA	ATCTCCCTA	1980
AGCGCCGTAA	CACCCCTGG	GACACGGGAT	GCTCCAAAT	GGCTTGTGC	CCCTGCTACG	2040
ACCTCTCAA	AGTATCCAAT	TCCTTCAAG	GGGCTACTCG	AGGGGGCAGA	TGCAACCCCTC	2100
TAGTCTCTAGA	ATTCACTGAT	GCAGGAAAAA	AGGCTAATTG	GGACGGGCC	AAATCGTGGG	2160
GACTGAGACT	GTACCGGACA	GGAACAGATC	CTATTACCAT	GTTCTCCCTG	ACCCGCCAGG	2220
TCCTCAATAT	AGGGCCCGC	ATCCCCATTG	GGCTTAATCC	CGTGATCACT	GGTCAACTAC	2280
CCCCCTCCCG	ACCCGTGCA	ATCAGGCTCC	CCAGGCTCTC	TCAGCCTCCT	CCTACAGGCG	2340
CAGCCTCTAT	AGTCCCTGAG	ACTGCCAAC	CTTCTCAACA	ACCTGGGACG	GGAGACAGGC	2400
TGCTAAACCT	GGTAGAAGGA	GCCTATCAGG	CGCTTAACCT	CACCAATCC	GACAAGACCC	2460
AAGATTTG	GCTGTGCTTA	GTGTCGGGAC	CTCCTTATT	CAGAGGAGTA	GGGGCTGTGG	2520
GCACATTATAC	CAATCATTCT	ACCGCCCCGG	CCAGCTGTAC	GGCCACTTCC	CAACATAAGC	2580
TTACCCATAC	TGAAGTGACA	GGACAGGGCC	TATGCATGGG	AGCACTACCT	AAAACCTACC	2640
AGGCCTTATG	TAACACCACC	CAAAGTGCCG	GCTCAGGATC	CTACTACCTT	GCAGCACCCG	2700
CTGGAACAAAT	GTGGGCTTGT	AGCACTGGAT	TGACTCCCTG	CTTGTCCACC	ACGATGCTCA	2760
ATCTAACACC	AGACTATTGT	GTATTAGTGT	AGCTCTGGCC	CAGAATAATT	TACCACTCCC	2820
CCGATTATAT	GTATGGTCAG	CTTGAACAGC	GTACCAAATA	TAAGAGGGAG	CCAGTATCGT	2880
TGACCCCTGGC	CTTTCTGCTA	GGAGGATTA	CCATGGGAGG	GATTGCAGGT	GGAAATAGGGA	2940
CGGGGACCC	TGCCCTAATC	AAAACCCAGC	AGTTTGGAC	GCTTCACGCC	GCTATCCAGA	3000
CAGACCTCAA	CGAAGTCGAA	AAATCAATT	CCAACCTAGA	AAAGTCACTG	ACCTCGTTG	3060
CTGAAGTAGT	CCTACAGAAC	CGAAGAGGCC	TAGATTGCT	CTTCCTAAA	GAGGGAGGTC	3120
TCTGCCAGC	CTAAAAGAA	GAATGTTGT	TTTATGCA	CCACACGGGA	CTAGTGAGAG	3180
ACAGCATGGC	CAAACCTAAGG	GAAGGCTTA	ATCAGAGACA	AAAACATT	GAGTCAGGCC	3240
AAGGTTGGTT	CGAAGGGCAG	TTTAATAGAT	CCCCCTGGTT	TACCACTTA	ATCTCCACCA	3300
TCATGGGAC	TCTAATAGTA	CTCTTACTGA	TCTTACTCTT	TGGACCCCTG	ATTCTCAATC	3360
GATTAGTTCA	ATTGTTAAA	GACAGGATCT	CACTAGTCCA	GGCTTTAGTC	CTGACTCAAC	3420
AATACCACCA	GCTAAAGCCT	ATAGAGTACG	AGCCATAGGG	CGCCTAGTGT	TGACAATTAA	3480
TCATGGCAT	AGTATACGGC	ATAGTATAAT	ACGACTCACT	ATAGGAGGGC	CACCATGGCC	3540
AAGTTGACCA	GTGCCGTTCC	GGTGCTCAC	GGCGCCGAGC	TCGCCGGAGC	GGTCGAGTTC	3600
TGGACCCGACC	GGCTCGGGGT	CTCCCCGGAC	TTCTGTTGAGG	ACGACTTCGC	GGGTGTGGTC	3660
CGGGACGACG	TGACCCCTGTT	CATCAGCGCG	GTCCAGGAC	AGGTGGTGC	GGACAAACACC	3720
CTGGCCTGGG	TGTGGGTGCG	CGGCCCTGGAC	GAGCTGTACG	CCGAGTGGTC	GGAGGTCGTG	3780
TCCACGAAC	TCCGGGACGC	CTCCGGGCCG	GCCATGACCG	AGATGGCGA	GCAGCCGTGG	3840
GGGGGGGAGT	TCGCCCTGCG	CGACCCGGCC	GGCAACTGCG	TGCACTTCGT	GGCCGAGGAG	3900
CAGGACTGAN	NNNCGGACCG	GTCGA				3925

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 96/02061	
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A. CLASSIFICATION OF SUBJECT MATTER			
IPC 6	C12N15/86	C12N5/10	C12N15/67

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF VIROLOGY 69 (7). 1995. 4086-4094. ISSN: 0022-538X, July 1995, XP002023654 LUUKKONEN B G M ET AL: "Efficiency of reinitiation of translation on human immunodeficiency virus type 1 mRNAs is determined by the length of the upstream open reading frame and by intercistronic distance." see the whole document --- VIROLOGY (1995), 208(1), 215-25 CODEN: VIRLAX; ISSN: 0042-6822, 1 April 1995, XP002023655 HERZOG, ETIENNE ET AL: "Translation of the second gene of peanut clump virus RNA 2 occurs by leaky scanning in vitro" see the whole document --- -/-	1-29
A		1-29

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- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentiaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Hornig, H

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/02061

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